Vol. 15 No. 2

# PLANT PHYSIOLOGY

APRIL, 1940

## FACTORS AFFECTING REGENERATION OF THE HORSERADISH ROOT[1]

ROBERT C. LINDNER

(WITH TWELVE FIGURES)

### Introduction

The term "regeneration" is ordinarily used to indicate the formation anew of a part of an organism that has been lost. In this paper the term is applied to the initiation of buds and roots from isolated pieces of the horseradish root. The growth of any plant axis, whether it be root or stem, may be separated into at least two phases: (1) The actual initiation of a growing point from a mass of meristematic tissue, and (2) the maintenance and subsequent development of this growing point. In the work reported here, no attempt was made to analyze the factors involved in the subsequent development of buds and roots after they were initiated. Reviews of the older literature on regeneration may be found elsewhere (**9, 12, 15, 16**).

The horseradish, *Cochlearia armoracia*, is a convenient plant for a study of this type, because small isolated pieces of the root regenerate both buds and roots quite readily when placed in the proper environment. Furthermore, these pieces are fairly resistant to invasion by microörganisms. Previous work (**13**) has shown that normally the initiation of buds and roots is confined to those regions of the root where a lateral root has been broken off. Both buds and roots apparently arise from identically the same region. It has also been shown that the application of relatively high concentrations of indoleacetic and naphthyleneacetic acids inhibits the development of buds and stimulates the development of roots. The purpose of this paper is to elucidate some of the factors involved in the initiation of buds and roots, with particular emphasis on bud initiation.

### Methods

Horseradish roots were obtained from a market in Chicago and stored at 2°–4° C. until used. If the humidity of the storage room is kept high

[1] Contributions from the Hull Botanical Laboratory no. 513.

enough to prevent excessive desiccation, the roots may be stored for months at this temperature without apparent detrimental results. Roots of approximately the same diameter (20–28 mm.) were used to insure more uniform test material. When a root was prepared for use, the upper and lower portions were discarded, leaving the central portion for experimental purposes (fig. 1). Adhering dirt was removed by scrubbing with a stiff-bristled brush

FIG. 1. Stages in preparation of horseradish root: (1) dormant root; (2) after trimming and washing; (3) after removal of test pieces. The ends of the root traces are visible as small elevations on the surface of the root.

while washing thoroughly with water. Care was taken to prevent injury to the root during this process. With a short cork-borer, a small piece of root was removed containing a root trace and its surrounding tissue. The piece was removed from the cork-borer by pressing on the end opposite the periderm with a glass rod (fig. 2A). A short cork-borer is advantageous in this procedure; the one used in most of the work was 6 mm. in diameter and 30

mm. long. After removal from the cork-borer, a test piece of the desired length (usually either 1 mm. or 3 mm.) was cut with a sharp razor, and the remaining tissue discarded. Ten such test pieces were placed with cut ends down on clean moist sand in a Petri dish (fig. 2B) and kept in the dark at

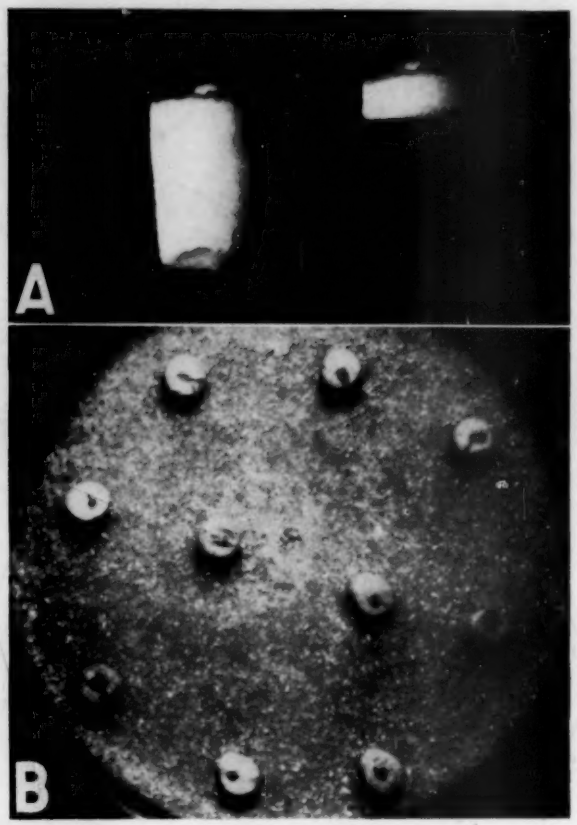



FIG. 2. Preparation of test pieces. A: piece on left as removed from cork-borer; piece on right trimmed to 3 mm. test piece (×2). B: test pieces on moist sand in Petri dish. The ends of the root traces are visible as small elevations on the surface of the pieces (natural size).

25° to 26° C. in an electric oven. The sand used in this work was washed successively with concentrated hydrochloric acid, tap water, and distilled water; it was then dried before use. About 23 ml. of dry sand were added to each Petri dish—an amount just sufficient to take up by capillarity 10 ml. of distilled water (or the solution to be tested). Under these conditions buds and roots first appear in about six days, and by nine days the maximum

number is formed. Observations were usually made after ten days with the aid of a dissecting microscope (fig. 3).

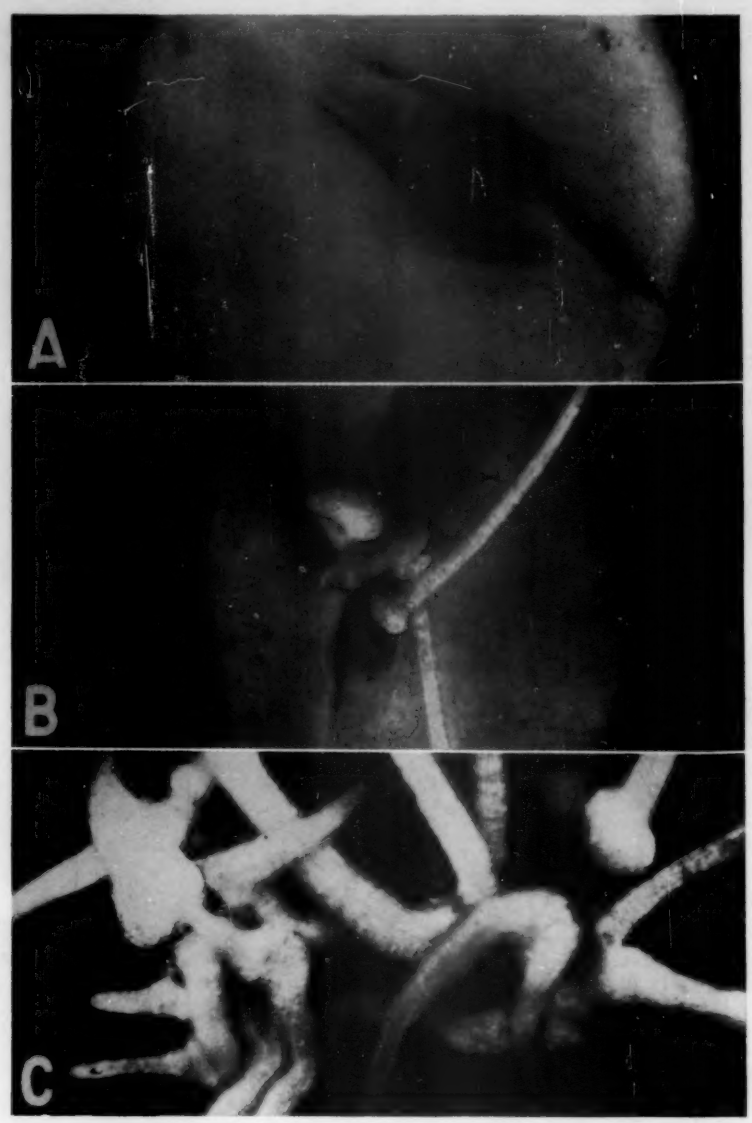



FIG. 3. Regeneration of test pieces. A: test piece at time of preparation. B: normal regeneration from test piece 3 mm. in length showing 2 roots and 3 buds. C: test piece treated with a 1 per cent. lanolin mixture of naphthyleneacetic acid showing formation of numerous roots (×15).

Since a bud or root is discernible at the surface of the test piece as soon as it becomes organized, the number of buds and roots formed may be used as a quantitative measure of regeneration. The number produced per ten test pieces was chosen as a standard measure, each Petri dish thus constituting a sample. Five replications or more were used, giving a population of at least fifty test pieces for any single determination. No attention was given to the relative size or vigor of an individual bud or root since only the factors involved in their initiation were being considered. The results reported in this paper were obtained from over 15,000 test pieces.

A major criticism of the method used was the lack of control of microorganisms. Most of the test pieces were able to resist invasion, however, if they were not damaged severely by the experimental treatment. Since the irregular surface of the piece made sterilization almost impossible, it was thought better to use a larger number of test pieces rather than introduce other variables in trying to obtain sterile cultures. Another problem was the introduction of chemical impurities in the sand which might affect the growth responses of the pieces. Such possible effects were minimized by thoroughly washing the sand with acid and water before use. No satisfactory substitute for sand was found that afforded adequate aeration and a good water supply. An additional problem was the past history of the material tested. If one were to grow the horseradishes under controlled conditions, take special precautions to see that they receive no injuries, and then rigorously select the roots, more uniform material would be obtained. Such a procedure is not feasible, and thus the best alternative appears to be the selection of roots of about the same size and the use of only those root traces that appear to be comparable. At any rate, it is not the actual number of buds and roots produced in any one test that is significant; it is the general trend of an experiment which results from a large total number of tests.

TABLE I

RELATION OF AREA OF TEST PIECE TO AMOUNT OF REGENERATION

| DIAMETER | AVERAGE NUMBER OF ROOTS PER TEN PIECES | AVERAGE NUMBER OF BUDS PER TEN PIECES |
|---|---|---|
| *mm.* | | |
| 3 | 16 ± 2* | 33 ± 2* |
| 6 | 19 2 | 32 2 |
| 9 | 17 2 | 35 2 |
| 12 | 39 3 | 70 4 |
| 15 | 37 3 | 67 4 |

* Standard deviation expressed to nearest whole number.

$$\sigma = \sqrt{\frac{\Sigma d^2}{n-1}}$$

## Experimentation

As a preliminary experiment, it was essential to determine the effect of the diameter of the test piece on initiation of buds and roots. Pieces 3 mm. long, varying in diameter from 3 mm. to 16 mm., were tested in the dark at 25° C. Owing to the distribution of the root traces in the root, only one root trace was included per test piece in pieces up to 9 mm. in diameter; above 9 mm. two root traces had to be included. The results, shown in table I, tend to indicate that only the root traces are important in bud and root initiation. For convenience in handling, the 6-mm. piece was chosen as the standard piece.

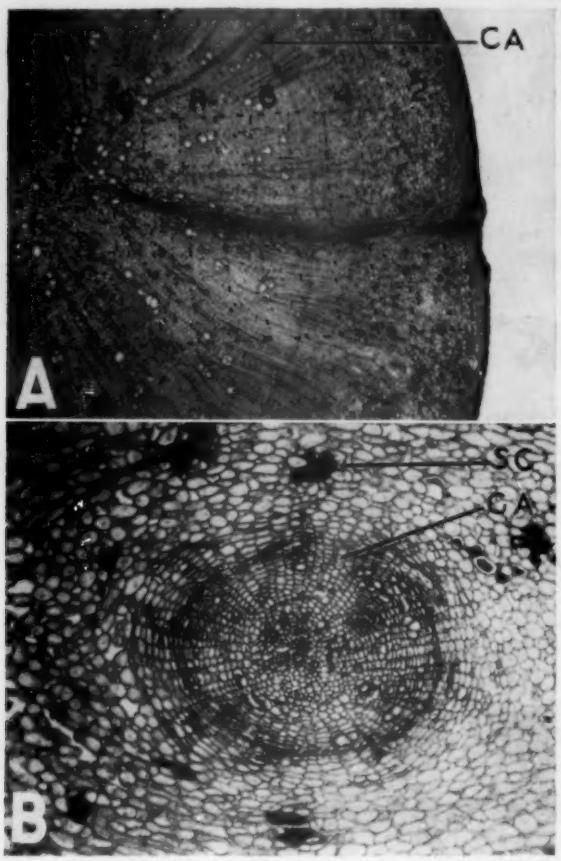



FIG. 4. Tissues associated with test piece. A: cross section of root 10 mm. in diameter showing longitudinal section of root trace. Diagram of test pieces imposed and drawn to scale as if the root were 24 mm. in diameter; ca, cambium (×15). B: tangential section through phloem portion of root showing cross section of root trace; ca, cambium; sc, stone cell (×150).

It was also necessary to know the relation of length of test piece to amount of regeneration. Pieces 6 mm. in diameter, but of lengths varying from 1 to 12 mm., were tested in the dark at 25° C. Figure 4 shows the tissues involved in these tests and the results are shown in figure 5. Al-

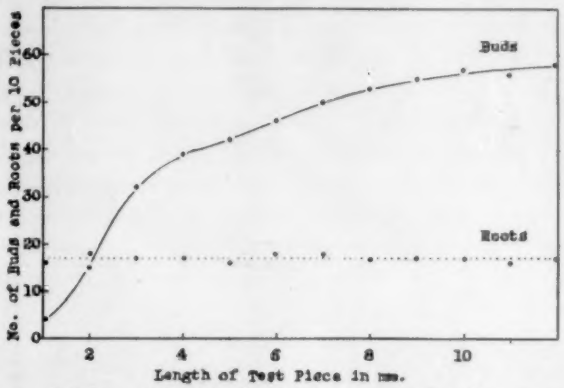



FIG. 5. Relation of length of test piece to amount of regeneration.

though the inner tissues apparently have no effect on the number of roots initiated, they do have a marked effect on the number of buds initiated.

Further evidence concerning the importance of the root trace for bud initiation was obtained by the following experiment. Pieces 6 mm. in diameter and 8 mm. long were used, and the root trace was severed by cutting a deep notch 1 mm. from the periderm. Others were ringed in the same place in order to sever nearly all tissue except the root trace. In all cases where the root trace was cut, the amount of regeneration corresponded to that of a piece 1 mm. long. On the other hand, when a root trace was left intact, the amount of regeneration corresponded to that of a piece 8 mm. long. In another experiment, pieces of various sizes were taken from parts of the root in which there were no root traces and tested in the dark at 25° C. In no case was there initiation of either roots or buds.

The question arose as to whether an inner portion of the test piece would initiate buds and roots if it were separated from the outer portion containing the periderm. Standard pieces 6 mm. in diameter and 8 mm. long were used; the outer 1 mm. consisting of periderm and outer phloem was discarded. In some cases the tissue produced a root or bud, more frequently a bud. If the outer 2-mm. piece was discarded, only buds were initiated, and these very rarely. These results seem to indicate that there is some factor, or factors, for both root and bud initiation in the outer 1 or 2 mm. of tissue.

It appears, therefore, that root and bud initiation are confined to the root trace. Only the outer tissues seem to be necessary for root initiation; for

maximum bud initiation, however, both inner and outer tissues seem to be necessary. Hereafter, for the sake of simplicity, the influence of the inner tissues in the stimulation of bud initiation will be referred to as the "bud factor." Furthermore, the term "factor" will be used in a broad sense to include any agent (physical, chemical, or otherwise) that produces an effect, and will not necessarily imply that a chemical entity is involved.

After the "bud factor" was shown to be confined to the root trace, it became desirable to know something about the rate of movement. Standard pieces 6 mm. long were divided into ten different lots. From one lot each day, the outer 1 mm. of tissue was removed from the pieces, and the inner 5 mm. were discarded. Observations were made on the fifteenth day. The results shown in figure 6 indicate that there is very little movement of the

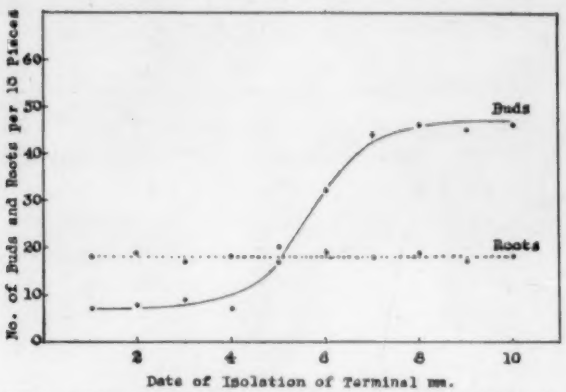



FIG. 6. Relation of time of isolation of 1 mm. test piece to amount of regeneration. The abscissa indicates time of isolation in days after beginning of experiment.

"bud factor" during the first four days of the experiment. Between the fifth and seventh days, however, there is a rather sudden movement. There are several ways of explaining these results. We may assume the "bud factor" to be present in the intact root, but we must then conclude that it does not move toward the periderm until at least four days after isolation of the test piece. On the other hand, the "bud factor" may not be present in the intact root and may be formed only about four days after isolation of the test piece. Another possibility is that the inner tissues merely act as a reservoir for the accumulation of bud inhibitors that are present in the outer tissues. These inhibitors may not move inward until after four days.

Since the "bud factor" has been shown to be transported along the root trace, some information regarding the diffusibility of this stimulus was sought. Standard test pieces 8 mm. long were used and the outer 1 mm. was removed with a clean, sharp razor. In some cases the two resulting pieces were immediately pressed tightly together, while in others a layer of

gelatin, agar, lanolin, or lens paper was placed between them. In every case the 1-mm. piece acted like an isolated 1-mm. piece. Apparently there was no diffusion of the "bud factor" across the break in continuity. These experiments were duplicated by attaching to the 1-mm. test piece a piece of inner tissue 7 mm. in length which had been killed with ether vapor (ether removed by aeration for six hours before pieces were used). In every case the 1-mm. test piece was killed, probably by the mustard oil or by other decomposition products of the dead tissue. There remained the possibility that if the "bud factor" were not present when the test piece was first isolated, it might not form unless it was under the influence of the developing primordia in the 1-mm. test piece. To test this possibility, the above experiments were duplicated, using test pieces that were severed 1, 2, 3, and 4 days after isolation. The same results were obtained, however, and in no case was there any evidence of bud stimulation. Thus one may conclude that the "bud factor" requires intact cells for its transfer.

Previous work has shown that when the horseradish root is cut into transverse segments about 4 cm. long, the development of buds and roots occurs in a polar manner; *i.e.*, buds develop at the morphological upper ends of the segments and roots at the morphological lower ends, regardless of the orientation of the segments during the period of regeneration (**13**). These observations were limited to gross development of both buds and roots and were not concerned with the actual initiation of growing points. Since there is this polarity in development, there might also be a polarity in the distribution of factors responsible for initiation. To test this possibility, transverse segments of the root, about 6 cm. long, were placed in a moist chamber on moist sand and maintained in the dark at 25° C. For nine successive days, standard test pieces, 6 mm. in diameter and 3 mm. in length, were removed from the tops and bottoms of these segments. Observations were made on the twelfth day. The results are shown in figure 7. The intact root appar-

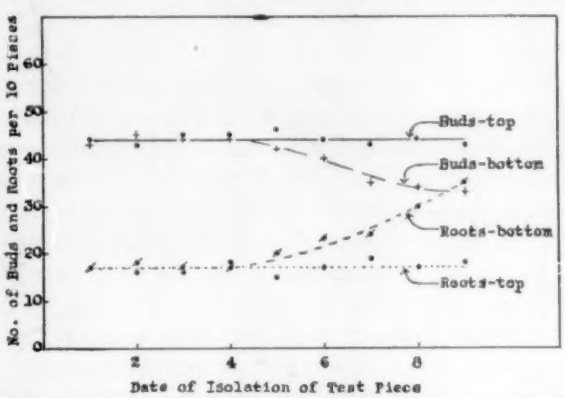



FIG. 7. Relation of polarity to initiation of buds and roots. The abscissa indicates time of isolation in days after beginning of experiment.

ently possesses no polarity in the distribution of the factors for bud and root initiation. The gradual increase in the number of roots and decrease in the number of buds, from the test pieces removed from the lower portions of the root segments, could be interpreted on the basis of the gradual accumulation in this region of some factor that stimulated root initiation and inhibited bud initiation. This factor might consist of the native auxins.

A partial analysis of the internal environment affecting bud and root initiation has shown that the inner tissues of the root trace stimulate bud initiation. This "bud factor" is transported along the root trace, is not diffusible, and is not polarly distributed. There are indications that it may be inactivated by one of the factors controlling root initiation.

Some of the relationships of the external environment to regeneration were next investigated. In order to determine the effects of variations in temperature, standard test pieces 6 mm. in diameter and 3 mm. in length were kept at various temperatures in the dark. Observations were made at the end of ten days. The results are shown in figure 8. The optimum

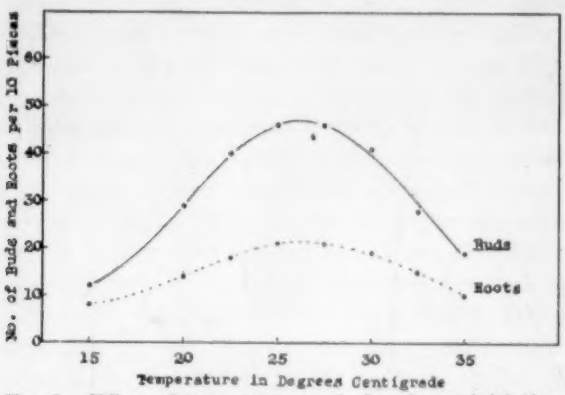



FIG. 8. Effect of temperature on bud and root initiation.

temperature for both root and bud initiation is about 26° C. Between 15° C. and 25° C. the $Q_{10}$ for bud initiation is 2.8, while that for root initiation is 1.6. This may indicate that the limiting process in bud initiation is a chemical reaction, while that for root initiation is a physical one.

Additional information concerning temperature effects was obtained through the following experiment. Ten groups of test pieces were placed at 20° C. and 25° C. respectively. On each day, for a period of 10 days, a group was changed from 20° to 25° C. and another group from 25° to 20° C. Observations were made at the end of 12 days. The results are shown in figure 9. They indicate that temperature affects the initiation of buds and roots only at a particular stage in their development; *i.e.*, between the sixth and eighth days after isolation. Previous work (**13**) has shown

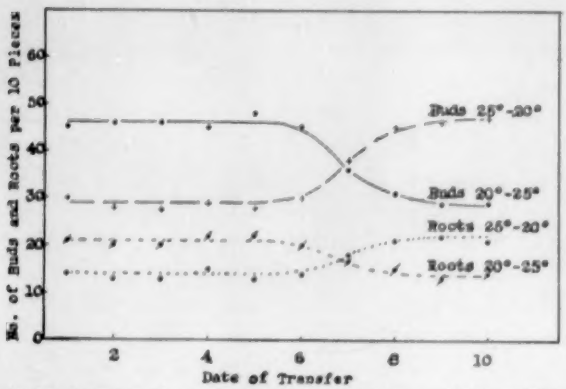



FIG. 9. Relation between time of temperature change and the initiation of buds and roots. The abscissa indicates time of transfer in days after beginning of experiment.

that this is the time when the meristematic regions are just beginning to organize into either buds or roots. It would appear, therefore, that temperature does not play an important rôle in the initial cell divisions, but is more effective in limiting the actual organizational processes.

A careful analysis of the effects of light on bud and root initiation was not attempted. A few experiments seemed to indicate that light stimulated bud production and inhibited root production. More exact experiments are necessary, however, to distinguish between the effects of light and the effects of temperature. To overcome any effects that light might have, all tests were made in the dark.

It was desirable to determine what effect the hydrogen-ion concentration of the medium around the test pieces would have on bud and root initiation. In these experiments, MCILVAINE'S standard buffer solutions (ranging from pH 2.2 to 8.0) were used instead of distilled water to moisten the sand in

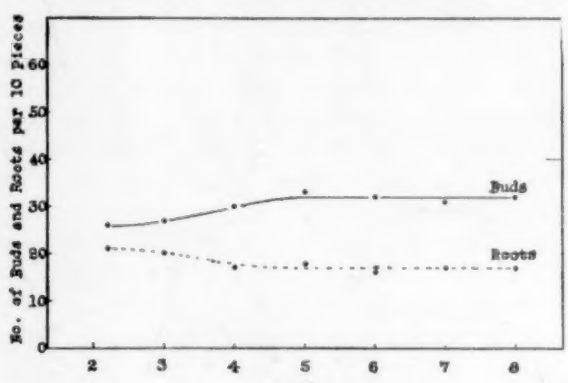



FIG. 10. Effect of hydrogen ion concentration on initiation of buds and roots.

the Petri dishes. These solutions (**4**), consisting of citric acid and potassium mono-hydrogen phosphate, are convenient to use because a wide range of pH may be obtained by varying the proportions of the two constituents. In every case the concentrations of the solutions were diluted one-tenth in order to prevent injury of the test pieces by high concentrations of salts. The hydrogen-ion concentration of the resulting solutions was checked with a quinhydrone electrode. No attempt was made to control the hydrogen-ion concentration of the tissue of the test pieces, and no determinations of tissue pH were made. The effects of these solutions on standard test pieces 3 mm. in length are shown in figure 10. Apparently the pH of the medium has little influence on the initiation of either buds or roots. This may be caused, in part, by a high buffer capacity of the tissue of the test piece. In the more acid solutions, there was a slight increase in number of roots and a slight decrease in number of buds. This could be accounted for most easily on the basis of an activation of native auxins by the acid (**2**).

After the method had been sufficiently standardized, it became of interest to determine the effect of specific chemical compounds on the initiation of buds and roots. In previous work, indoleacetic and naphthyleneacetic acids, in relatively high concentrations, were shown to be very effective in inhibiting the development of buds and in stimulating the development of roots (**13**). It was thus advisable to analyze further the effects of these compounds. Standard test pieces 3 mm. in length were treated with aqueous solutions of indoleacetic acid or naphthyleneacetic acid ranging in concentrations from $5.4 \times 10^{-4}$ M to $5.4 \times 10^{-14}$ M. Ten ml. of these solutions were added to the sand in Petri dishes. Since there were ten test pieces per dish, each piece had a maximum volume of 1 ml. of solution at its disposal, although only a small portion of this amount was probably absorbed. Lanolin mixtures of each of these substances were also applied to the cut surfaces of test pieces in concentrations ranging from $5.4 \times 10^{-2}$ M to $5.4 \times 10^{-7}$ M. These two different means of application gave essentially the same results. The effects of aqueous solutions of naphthyleneacetic acid are shown in figure 11. In the higher concentrations there is apparently a stimulation of root initiation and an inhibition of bud initiation (figure 3). No evidence was obtained for bud stimulation at any concentration over the wide range employed. The lower concentrations of naphthyleneacetic acid are more effective in bud inhibition than they are in root stimulation. Indoleacetic acid did not prove very effective, presumably because it was inactivated. The inactivation may have been caused by oxidizing enzymes from the test pieces, microörganisms, inorganic constituents of the sand, or a combination of these factors. At any rate, the "bud factor" appears to have no direct relation to the auxins; but it may be inactivated by them, or at least ineffective in their presence.

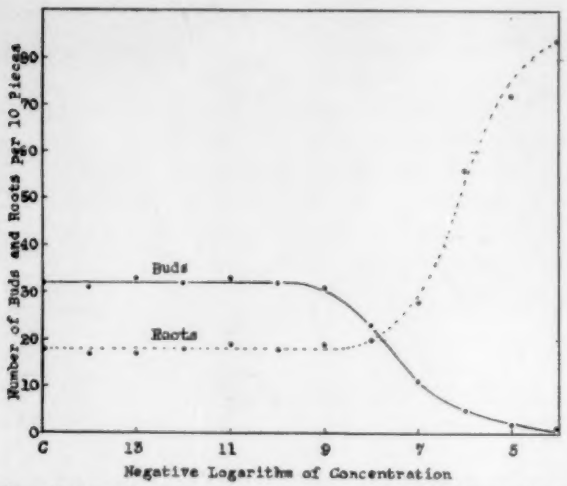



FIG. 11. The relationship of aqueous solutions of naphthyleneacetic acid to the initiation of buds and roots. The abscissa indicates the concentration in grams per liter. C, control.

When small isolated pieces of various tissues of the root (xylem only, phloem only, periderm plus phloem, etc.) were treated with relatively high concentrations of naphthyleneacetic acid, roots were initiated from all of the tissues. The main cambial region and the region of the phellogen were the most active in this respect. Root traces were not included in any of these pieces.

A number of standard test pieces, 3 mm. in length, were divided into twelve lots. Each day, for 12 days, a different lot was treated with a 1 per cent. lanolin mixture of naphthyleneacetic acid. Each lot thus received but one application. Observations were made after 20 days. The results are shown in figure 12. Apparently the number of roots initiated is about the same regardless of the time of application of naphthyleneacetic acid. The number of buds, however, is most affected if the treatment is made during the first four days after isolation. The application of naphthyleneacetic acid after this time was apparently too late to inhibit the initiation. This period between 5 and 7 days is the time when meristematic areas are just beginning to organize into either a bud or a root. Thus after a bud is once organized, it will not change over into a root although root initials may develop at the base of the bud. Gross observations of buds from pieces treated 6 days and more after isolation substantiate this conclusion (compare the results of the experiment shown in figure 7 in this regard). Between the fifth and eighth days, in this experiment, there was a maximum decrease in the number of buds on the test pieces isolated from the lower portions of the segments. This might indicate that the native auxins (or other bud

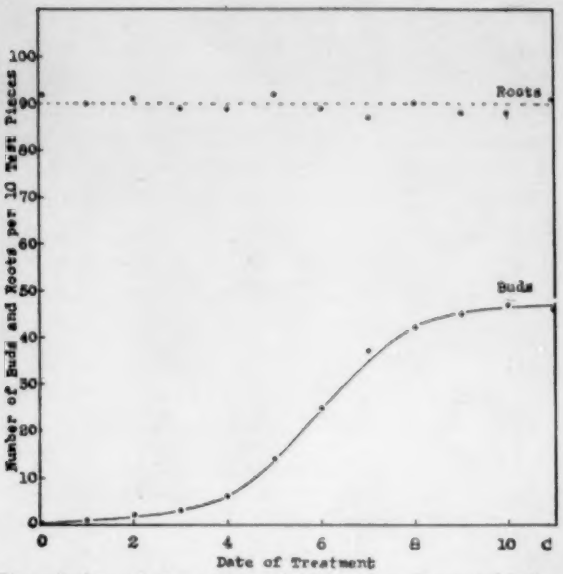



FIG. 12. The relation of time of application of a 1 per cent. lanolin mixture of naphthyleneacetic acid to the initiation of buds and roots. The abscissa indicates time of treatment in days after start of experiment. C, control.

inhibitors) do not start to accumulate in the lower regions of transverse segments of the root until about 4 days after isolation.

In the experiments reported in table I and in figures 5, 6, 10, and 11, the maximum number of buds formed per ten test pieces, 6 mm. in diameter and 3 mm. in length, is near 32; while for the experiments reported in figures 7, 8, 9, and 12, the maximum number is near 46. This is attributable to the fact that horseradish roots from different sources were used for these two different groups of experiments. The higher maximum was obtained on test pieces from roots purchased in the fall; the lower maximum was obtained on test pieces from roots purchased in the winter. Thus it is shown, in an indirect fashion, that the past history of the root is an important factor in determining the amount of regeneration.

After some of the effects of auxin-like compounds had been ascertained, the effects of other pure substances were determined. A number of inorganic and organic compounds were applied over a wide range of concentrations as aqueous solutions to the sand in the Petri dishes. Standard 1-mm. or 3-mm. test pieces were used in testing these substances at 25° C. in the dark. The following is a list of the materials tried:

| | |
|---|---|
| Ammonium sulphate | Calcium nitrate |
| Ascorbic acid | Chloral hydrate |
| Calcium chloride | Citric acid |

| | |
|---|---|
| Cupric sulphate | Pangestin |
| Dioxane | Papain |
| Eosin | Potassium chloride |
| Ethyl alcohol | Potassium cyanide |
| Ethylene | Potassium di-hydrogen phosphate |
| Ethylene chlorohydrin | Potassium nitrate |
| Ferric sulphate | Potassium permanganate |
| Ferrous sulphate | Saliva |
| Formaldehyde | Sodium taurocholate |
| Glycine | Theelin |
| Hematoxylin | Theelol |
| Hydrogen peroxide | Thiourea |
| Inositol | Tyrosine |
| Magnesium sulphate | Urea |
| Maleic acid | Urine |
| Manganous sulphate | Vitamin $B_1$ (thiamin) |
| Methyl amine | Vitamin $B_2$ |
| Methylene blue | Yeast extract (aqueous) |
| Nicotinic acid | Zinc sulphate |

In general, high concentrations were toxic, but other than this, no marked effects were produced. Thus it is probable that none of these substances alone limited the initiating processes.

Preliminary extracts from various tissues of the horseradish root have given some indication of being capable of stimulating bud initiation. These extracts were tested on standard 1-mm. pieces and at least four different concentrations of each extract were used; the highest concentration was usually toxic. The following is an outline of the extracts tried:

I. Inner tissues
   1. Ether extract
   2. Cell sap

II. Outer and inner tissues
   1. Water extract
   2. Fraction of water extract soluble in 70 per cent. alcohol
   3. Fraction of water extract insoluble in 70 per cent. alcohol

III. Inner tissues from roots allowed to regenerate 6 days
   1. Ether extract
   2. Water extract
   3. Cell sap
   4. Water diffusate
   5. Water extract[2] following previous ether and water extracts

[2] These extracts stimulated bud initiation at least 300 per cent.

6. Cell sap from frozen tissue
   a. Soluble fraction not passing through collodion membrane
   b. Insoluble fraction not passing through collodion membrane

IV. Outer tissues from roots allowed to regenerate 6 days
1. Ether extract
2. Water extract[2] following previous ether extract
3. Cell sap from frozen tissue
   a. Soluble fraction not passing through collodion membrane
   b. Insoluble fraction not passing through collodion membrane

V. Outer and inner tissues from roots allowed to regenerate 6 days
1. Water extract
2. Fraction of water extract soluble in 70 per cent. alcohol
3. Fraction of water extract insoluble in 70 per cent. alcohol

One encounters many difficulties in trying to obtain active extracts since there may be many different types of inhibitors present, and inactivation of an extract may take place for many reasons. The results, however, indicate that the "bud factor" is a chemical entity. This may be more apparent than real since the extracts may be effective by destroying bud inhibitors (such as the auxins) and may be entirely independent of the "bud factor." Further work of this type is now in progress.

## Discussion

From the experiments reported here, it is apparent that the various tissues of the horseradish root possess different capacities for influencing the regeneration processes. Buds and roots are initiated only in association with a lateral root trace. The outer portions of the root trace appear to be responsible for root initiation; for maximum bud initiation, however, both the inner and outer portions are necessary. PRIESTLEY and SWINGLE (**15**) review some work on sea kale in this regard. The xylem tissues of the root of this plant, when isolated, produce buds from any of the cut surfaces, but do not form roots. The extra-cambial tissue, on the other hand, produces roots, but no buds. Thus these two species of plants are similar in that the inner tissues contain factors for bud initiation, and the outer tissues, factors for root initiation. They differ in that the horseradish regenerates only in association with a root trace, while any of the tissues of the sea kale root may regenerate. Furthermore, the horseradish requires factors that are present in the outer tissues of a root trace for maximum bud initiation.

The analysis of polarity phenomena in the horseradish indicates that in the dormant root there is no polar distribution of factors that cause either root or bud initiation. If the root is placed in an environment favorable for regeneration, however, the factors causing root initiation seem to become

polarly distributed. The factors associated with bud initiation also apparently become polarly distributed; this might be interpreted as caused by the inactivation of bud-initiating factors in the lower portions of the root or its isolated segments. SCHWANITZ (**18**) has done some work on this subject. He reports that intact rhizomes of *Lathyrus pratensis* and *Agropyrum repens* show a marked polar development of buds when allowed to regenerate. If the rhizomes are cut into transverse segments at the beginning of the experiment, each piece produces as many buds as any other piece. If severed on successive days, a gradual establishment of polarity is shown by the differential amount of bud development of the transverse segments. Further evidence has been accumulated by JONES (**11**), who reports that when segments of sea kale roots 5 to 10 cm. in length are centrifuged at least 3 days (1000 r.p.m.) with the apex of the segments away from the center, buds are usually produced from the base of the segments as well as at the apex. If segments are centrifuged with the base away from the center, however, they regenerate in a normal manner, *i.e.*, buds at apex and roots at base. This might mean that certain bud-inhibiting substances (such as auxins) are not subject to polar distribution when under the influence of an opposing centrifugal force, and thus their diffuse distribution allows buds to form from the basal ends of the segments. JONES also reports that segments of sea kale roots, 2 mm. or less in length, produced buds on both ends, while longer segments produced buds only on the upper end. This might indicate that the longer segments have more bud inhibitors than the shorter. STOUGHTON and PLANT (**20**), using segments of sea kale roots 7 cm. in length, cut off 1 mm. of tissue from the base and apex every 5 days for a period of 8 weeks. They obtained buds on both ends of 25 per cent. of the segments. This work would indicate that bud-inhibiting substances were removed when the end tissues were removed. FISCHNICH (**6**) was able to produce buds from callus at the basal end of poplar cuttings, that would ordinarily have produced roots, by ringing the cutting above the callus. The ringing supposedly prevented the accumulation of auxins in the basal callus.

In the horseradish, temperature has more effect on bud initiation than on root initiation. Other workers have reported similar results in regard to the relation of temperature to bud growth. CLARK (**3**) has shown that the decapitated hypocotyls of seedling tomato plants do not form buds from the cut surface unless the temperature is above 20° C. SIMON (**19**) reported that the development of buds from the callus of poplar cuttings was better at 32° C. than it was at 26° C. A temperature of 18° C. resulted in the formation of very little callus and inhibited the development of buds. JONES (**11**) found that a local increase of temperature as little as 2° C. induced the formation of buds on the lower ends of sea kale segments where roots ordi-

narily would have developed. Temperature is thus an important factor in bud initiation.

The hydrogen-ion concentration of the medium had little effect on bud and root initiation in the horseradish. JONES (**11**) reports similar results for sea kale. BONNER'S analysis (**2**) of the effects of hydrogen ions on the Avena coleoptile shows that the effects of acids are produced through the activation of auxins. RIEHM'S results (**17**) are a little difficult to understand in this regard. When he floated leaf fragments of *Cardamine pratensis* on a solution of $KH_2PO_4$, buds were produced in 3 days and roots in 5 days. When the leaf fragments were floated on $K_2HPO_4$, however, roots were formed in 3 days, but buds were not formed until after 14 days.

In the horseradish, the application of naphthyleneacetic acid over a wide range of concentrations inhibits the initiation of buds and stimulates the initiation of roots. STOUGHTON and PLANT (**20**) obtained similar results with sea kale roots by using relatively high concentrations of indoleacetic acid. WENT (**24**) also reports similar results with the roots of *Oenothera macrosiphon*. Thus it seems that, in certain plants at least, the initiation of buds from roots is inhibited by auxin-like compounds, while the initiation of roots is stimulated. The effects of these compounds on bud initiation from stems, however, are not so consistent. LINK and EGGERS (**14**) report inhibition of bud initiation from decapitated flax hypocotyls by application of relatively high concentrations of indoleacetic acid. On the other hand, GREENLEAF (**8**) reports a stimulation of bud initiation from the callus of decapitated tobacco plants through the use of a 1 per cent. indoleacetic acid mixture in lanolin. Likewise, GOLDBERG (**7**) reports similar results for cabbage seedlings. BEAL (**1**) obtained axillary buds by treating decapitated stems of *Lilium harrisii* with relatively high concentrations of indoleacetic acid. A closely related species, *L. longiflorum*, produced only roots in response to the treatment. HOWARD (**10**) reports that decapitated kale plants produce both buds and roots when treated with indoleacetic acid, but the buds are formed later than the roots—presumably after the supply of indoleacetic acid is exhausted. The work of COOPER (**5**), STUART (**21**), and WENT (**23**) on the mobilizing action of auxin-like compounds may lead the way to a solution of these problems in the future.

Ascorbic acid, thiamin, ethylene, and other "active" compounds are not effective in stimulating the initiation of either buds or roots in the horseradish. Extracts, however, indicate that the "bud factor" is a chemical entity. A factor affecting the development of buds has been reported by WENT (**22**). It is probably formed in the roots, requires intact cells for its transfer, and functions in bud elongation in collaboration with auxin. WENT has named this factor "caulocaline." Whether this factor is involved in bud initiation is not known.

## Summary

1. A method of analyzing some of the factors associated with root and bud initiation, by the use of small isolated pieces of the horseradish root, is outlined. The results from more than 15,000 test pieces are reported.

2. The various tissues of the horseradish root apparently possess different capacities for influencing the regeneration processes. Root and bud initiation are confined to the lateral root traces. The outer tissues of the root trace are apparently responsible for root initiation; for maximum bud initiation, both outer and inner tissues are necessary.

3. The factor (or factors) in the inner tissues that stimulates bud initiation moves only along the root trace, and requires intact cells for its transfer.

4. The dormant root of the horseradish does not possess a polar distribution of either root or bud initiation factors. A polarity in root initiation is established only after the root is placed in a favorable environment for regeneration. An apparent polarity in bud initiation is established at the same time.

5. Variations in temperature have more effect on bud initiation than on root initiation. Between 15° C. and 25° C. the $Q_{10}$ for bud initiation is about 2.8; that for root initiation is about 1.6. The optimum temperature for both bud and root initiation is about 26° C. Temperature produces its greatest effect just at the time the meristematic regions are organizing into buds or roots.

6. The hydrogen-ion concentration of the medium has little effect on either root or bud initiation.

7. Naphthyleneacetic acid, over a wide range of concentrations, inhibits bud initiation and stimulates root initiation. At no concentration is there any evidence of the stimulation of bud initiation. Indoleacetic acid was not very effective, presumably because it was inactivated. Relatively high concentrations of these substances are capable of inducing root initiation from isolated pieces of any living tissue of the root.

8. Numerous organic and inorganic compounds, including various vitamins and other "active" substances, had little effect on either root or bud initiation.

9. Tests with extracts of various parts of the horseradish root indicate that one of the factors present in the inner tissues that stimulates bud initiation is a chemical entity.

The writer is indebted to members of the Department of Botany of the University of Chicago for helpful suggestions made during the course of this study.

THE UNIVERSITY OF CHICAGO

## LITERATURE CITED


1. Beal, J. M. Histological responses of three species of Lilium to indoleacetic acid. Bot. Gaz. **99**: 881–911. 1938.
2. Bonner, James. The relation of hydrogen ions to the growth rate of the Avena coleoptile. Protoplasma **21**: 406–423. 1934.
3. Clark, Fred R. Temperature as a possible factor in regeneration. Plant Physiol. **6**: 196. 1931.
4. Clark, W. M. The determination of hydrogen ions. Williams and Wilkins, Baltimore. 1928.
5. Cooper, William C. Transport of root-forming hormone in woody cuttings. Plant Physiol. **11**: 779–793. 1936.
6. Fischnich, O. Die Rolle des Wuchstoffes bei der Bildung von Adventivsprossen und -wurzeln. Ber. d. bot. Ges. **56**: 144–152. 1938.
7. Goldberg, Ethel. Histological responses of cabbage plants grown at different levels of nitrogen nutrition to indole(3)acetic acid. Bot. Gaz. **100**: 347–369. 1938.
8. Greenleaf, Walter H. Induction of polyploidy in Nicotiana. Science n.s. **86**: 565–566. 1937.
9. Holm, Theodore. On the development of buds upon roots and leaves. Ann. Bot. **39**: 867–881. 1925.
10. Howard, H. W. Possible action of phytohormones as root-determiners. Ann. Bot. **2**: 933–942. 1938.
11. Jones, W. N. Polarity phenomena in sea kale roots. Ann. Bot. **39**: 359–372. 1925.
12. Krenke, N. P. Wundkompensation, Transplantion und Chimären bei Pflanzen. Transl. by N. Busch and O. Moritz. Julius Springer, Berlin. 1933.
13. Lindner, Robert C. Effects of indoleacetic acid and naphthyleneacetic acids on development of buds and roots in the horseradish. Bot. Gaz. **100**: 500–527. 1939.
14. Link, George K. K., and Eggers, Virginia. Inhibition of adventitious bud initiation in hypocotyl of flax by indole-3-acetic acid and flax extract. Nature **142**: 398–399. 1938.
15. Priestley, J. H., and Swingle, Charles F. Vegetative propagation from the standpoint of plant anatomy. U.S.D.A. Tech. Bull. 151. 1929.
16. Rauh, Werner. Die Bildung von Hypocotyl und Wurzelsprossen und ihre Bedeutung für die Wuchsformen der Pflanzen. Nova Acta Leopoldina **4**: 396–457. 1937.
17. Riehm, E. Beobachtungen an isolierten Blättern. Zeitschr. Naturw. **77**: 281–314. 1905.

18. SCHWANITZ, F. Beiträge zur Analyse der pflanzlichen Polarität. Beih. bot. Centralbl. **54A**: 520–530. 1936.
19. SIMON, S. Experimentelle Untersuchungen über die Differenzierungsvorgänge im Callusgewebe von Holzwächsen. Jahrb. wiss. Bot. **45**: 351–478. 1908.
20. STOUGHTON, R. H., and PLANT, W. Regeneration of root cuttings as influenced by plant hormones. Nature **142**: 293–294. 1938.
21. STUART, NEIL W. Nitrogen and carbohydrate metabolism of kidney bean cuttings as affected by treatment with indoleacetic acid. Bot. Gaz. **100**: 298–311. 1938.
22. WENT, F. W. Specific factors other than auxin affecting growth and root formation. Plant Physiol. **13**: 55–80. 1938.
23. ———. The dual effect of auxin on root formation. Amer. Jour. Bot. **26**: 24–29. 1939.
24. ———. Some experiments on bud growth. Amer. Jour. Bot. **26**: 109–117. 1939.

# THE ABSORPTION OF CARBON DIOXIDE BY UNILLUMINATED LEAVES

JAMES H. C. SMITH

(WITH ELEVEN FIGURES)

## Introduction

In the process of photosynthesis as carried out by the higher plants, the leaf absorbs carbon dioxide from the air and transforms it into organic matter. This assimilation of carbon dioxide is accomplished through a number of intermediate steps and any complete analysis of the mechanism of this process requires that the carbon be traced through these consecutive steps. Obviously the first step is the absorption of carbon dioxide from the air.

The critical experiments of BLACKMAN (**4**) demonstrated that the carbon dioxide of the air enters the leaf through the stomata. At first this view was difficult to accept because it seemed impossible that the volume of carbon dioxide necessary to maintain active photosynthesis could diffuse through such a small area. The stomatal area in the sunflower leaf is only 3.75 per cent. of the total.

This difficulty was removed, however, when BROWN and ESCOMBE (**5, 6**) showed that the diffusion of a gas through a perforated septum is almost unobstructed if the apertures have the proper dimensions and distribution. Examination showed that the required conditions are fulfilled by the stomata of the sunflower leaf, and that the amount of carbon dioxide necessary to maintain the maximum rate of photosynthesis so far observed can easily diffuse into the leaf provided "the interior of the leaf were a perfect 'sink' of atmospheric carbon dioxide." These small openings have the power of "drinking in atmospheric carbon dioxide" about fifty times faster than apertures of like dimensions filled with "a constantly renewed solution of alkali hydroxide."

This exceptional ability to "drink in" carbon dioxide led WILLSTÄTTER and STOLL (**33a**) to search for the carbon dioxide absorptive agent in leaves. Their researches (**33b**) showed that some leaves, particularly sunflower and nettle leaves, even when unilluminated, absorb reversibly considerable quantities of carbon dioxide. This absorption is not a function of the life process of the leaves. Probably the green pigments of the leaf have nothing to do with this reaction because yellow varieties of certain species absorb carbon dioxide as well as the green varieties. WILLSTÄTTER and STOLL (**33c**) isolated no substances from leaves which could account for the carbon dioxide combining capacity. They suggested that alkali and alkaline earth bicarbonates, especially magnesium bicarbonate, might be the source of the

reversible absorption. They were more inclined to the view, however, that the chief absorptive agents are the amino compounds which react to form carbamino acids.

WILLSTÄTTER and STOLL (33d) also measured the absorption of carbon dioxide by chlorophyll both in true solution and in colloidal suspension. From these measurements they concluded that chlorophyll, molecularly dispersed in alcohol, absorbed no carbon dioxide but that when colloidally dispersed in water it absorbed reversibly a small but definite quantity of this gas in addition to that absorbed by the magnesium which was split from the pigment as magnesium bicarbonate. They performed experiments to determine whether the carbon dioxide absorbed by the chlorophyll could be reduced to formaldehyde with the simultaneous production of a peroxide (33e). In every case the results were negative and the conclusion was reached that the illumination of chlorophyll in an atmosphere of carbon dioxide was not sufficient of itself to produce photosynthesis (33f).

In view of the importance of carbon dioxide absorption to photosynthesis and the fact that the active agent for the absorption of carbon dioxide by leaves had not been identified, SPOEHR and MCGEE (24, 25, 26) undertook to establish the nature of the absorbing substance in leaves. Their experiments demonstrated that dried leaf material absorbed carbon dioxide; that the chlorophyll could be extracted with acetone without affecting the absorption; that an absorbing agent could be extracted from dried sunflower leaves by means of water saturated with ether; and, that the dissolved material maintained quantitatively the absorption capacity removed from the leaf material. Because of the removal of the absorptive material from leaves by the CHIBNALL-SCHRYVER method for protein extraction (water saturated with ether) SPOEHR and MCGEE were inclined to the view that a proteinaceous substance was responsible for this absorption through the carbamino reaction.

Further investigations by SPOEHR and NEWTON (27, 28) established that the absorptive material could be precipitated from the water extract of sunflower leaves by the addition of alcohol, and that this material was diffusible through an animal membrane. This material did not contain enough amino-nitrogen or total nitrogen to account for the absorption of carbon dioxide in equivalent molecular proportions. Consequently the hypothesis of the carbamino reaction as the source of the absorption was no longer tenable. These experiments also showed that "the larger part of the absorption of carbon dioxide by dried leaf material and the alcoholic precipitates obtained therefrom could be ascribed to bicarbonate formation."

Comparison showed that the leaves from sunflower and nettle possessed the highest carbon dioxide absorptive capacity of all the leaves studied. In fact dried leaf material from spinach, hydrangea, turnip, alfalfa, rhu-

barb, and grass absorbed very little more carbon dioxide than could be accounted for by the water added. The final conclusion of SPOEHR and NEWTON was that possibly all leaves possess an absorptive capacity for carbon dioxide but that certainly for many it is very small.

Later SPOEHR (**23**) pointed out that special experiments would be required to demonstrate whether or not this absorption had any connection with the photosynthetic process, but that it is suggestive that both sunflower and nettle leaves possess high absorptive capacity for carbon dioxide and high photosynthetic activity.

In spite of the attempts made to establish the chemical system whereby carbon dioxide is absorbed by certain leaves, no definite knowledge of the constituents responsible for this reaction had been gained; nor had it been determined whether this reaction was in any way related to the photosynthetic process. Because of the importance of these questions, both explicit and implied, renewed attempts have been made to analyze the process whereby carbon dioxide is absorbed by the unilluminated leaf (**29**). For this purpose the carbon dioxide absorptive capacity of living and killed leaves, of chlorophyllous and nonchlorophyllous leaves, and of petals, roots, and leaves have been compared. The absorption of carbon dioxide by fractions (differing in solubility) from the sunflower leaves and by the chemical substances obtained therefrom has also been measured.

From these measurements it has become evident that leaves possess a very complex system for the absorption of carbon dioxide. This system is composed of several interdependent chemical reactions which are in equilibrium with the carbon dioxide of the atmosphere. This system might provide a reservoir of carbon dioxide which would be available to the photosynthetic process.

## Experimental procedure

### APPARATUS FOR MEASURING THE AMOUNT OF CARBON DIOXIDE ABSORBED

The apparatus used for measuring the absorption of carbon dioxide (fig. 1) consisted of two parts: I, a manometric system for measuring the absorption of the gas; and II, a pumping system for removing, collecting, and analyzing the gas from the reaction vessel.

ABSORPTION APPARATUS (I).—A gas reservoir G could be filled with the gas whose absorption was to be measured. After evacuation of G by means of a Hyvac or a mercury diffusion pump through stopcocks B and H, it was filled from a gas tank attached through stopcock I. The pressure in the reservoir G was measured by manometer F. Manometer D was included so that the pressure in reservoir G could be measured without altering its volume. Manometer D indicated when the pressures in G and F were equal. Equality of pressures in G and F was obtained by evacuation

through H and adjustment of the height of the levelling-bulb attached to A. Capillary tubes C and E served as safety tubes for the evacuation of manometer D. Capillary tube J connecting reservoir G and reaction flask L was long enough to permit the shaking of L. Stopcocks K and M served to connect the reaction flask either to the reservoir G or to the pumping system

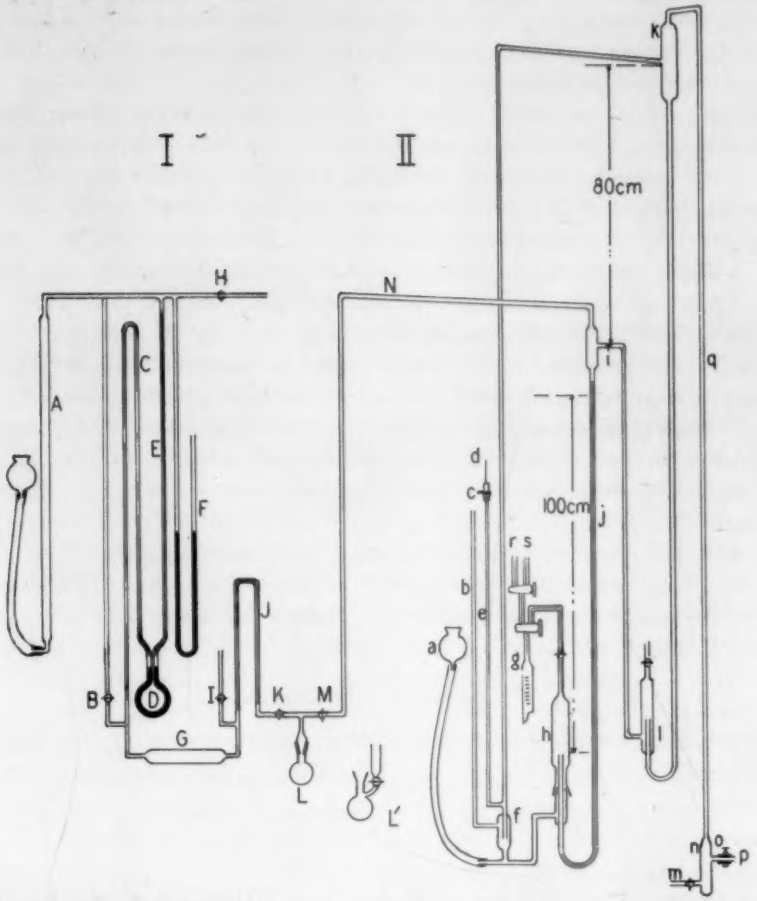



FIG. 1. Apparatus for measuring the absorption of carbon dioxide by leaf material. (I), absorption system. (II), pumping and gas-analysis apparatus.

(II). Reaction flask L′ equipped with an addition funnel could be substituted for reaction flask L when desired.

The gas reservoir G and the absorption flask L were submerged in a constant-temperature water bath. All tubing, C and J, outside of the water bath and included in the gas-measuring system was capillary tubing. This comprised about 3 per cent. of the total volume. Approximately a 0.1 per

cent. change in volume was caused by a change of 10° C. in room temperature. Under the experimental conditions used, error from this source was negligible.

APPARATUS FOR COLLECTING AND ANALYZING THE GAS (II).—The gas was pumped from the absorption flask L by means of a Sprengel pump, collected in a gas reservoir *h*, and transferred to a Hempel gas-analysis apparatus *g* for analysis. Descriptions of the Sprengel pump are given in manuals of laboratory practice (**7, 19**). The design shown here was better suited to our purpose than the ones more commonly used.

An air lift carried mercury from reservoir *f* to chamber *k*. The air lift was activated by a water aspirator attached at *p*. Screw clamp *o* regulated the flow of air to the aspirator. The proper mercury level was maintained in *f* by the levelling-bulb *a*. Screw clamp *c* regulated the flow of air through capillary *d*. The mercury, separated from the air in chamber *k*, flowed in a fine stream through nozzle *i* trapping the gas in capillary *j* and removing it from the absorption apparatus.

The gas receiver *h* was connected through a ground-glass joint so that it could be removed easily for cleaning. The air inlets to *f* were furnished with long tubes *b* and *e* so as to prevent the spilling of mercury when levelling bulb *a* was raised. The gas trap *l* was inserted to eliminate the sweeping of small extraneous gas bubbles into receiver *h*. Mercury which spattered into tube *q* was collected in receptacle *n* and removed through stopcock *m*.

Only a few critical dimensions needed to be regarded for the proper operation of the pump. Two of these are marked on the drawing. The distance between the nozzle *i* and the inlet into *k* had to be greater than the height of the column of mercury supported by the pressure in the tube N, which for our purpose was greater than 760 mm. The height of the column of mercury in the capillary tube *j* was also great enough to insure a sufficient rate of flow of mercury, in this instance a height of about 100 to 110 cm. The diameter of the capillary *j* (approximately 1 mm.) was sufficiently large to permit a rapid flow of mercury but small enough to prevent the trapped air from eddying past the flowing mercury. The opening of nozzle *i* had to be adjusted empirically to meet the other demands of the apparatus. This was done by constricting the tube to the desired diameter (approximately 1.5 mm.) by means of a hand torch.

The conversion factor for transforming pressure change in reservoir G to volume of gas absorbed in vessel L was obtained by measuring the change of pressure in reservoir G caused by removal of known volumes of gas. These volumes were measured in buret *g*. The reproducibility of these measurements was found to be about ± 0.1 ml.

Any change in the volume of the system KJGC required a redetermina-

tion of the value for the conversion factor. The value of this factor was not dependent on the volume of the reaction flask L. The reaction vessels were approximately 50-ml. capacity and the volume of KJGC was 122.7 ml. in many of the experiments reported.

PROCEDURE.—A standard procedure was adopted for measuring the absorption of carbon dioxide by the various materials which were placed in the reaction flask L, or L′. The flask was attached to the apparatus through the ground-glass joint and the material allowed to come to temperature equilibrium with the bath. Living leaves were stored an additional length of time, one or two hours, before measurements were begun so as to lessen their respiration.

Next, the flask was evacuated by means of the Sprengel pump. For nonliving material the pumping was discontinued when gas bubbles ceased to appear in the capillary tube *j*. This point was easily determined. For living material, however, gas continued to be removed even after long periods of pumping. For such material an arbitrary period of twenty minutes was set, since this had been shown to be ample time to remove all gas from the empty reaction vessel.

The space in the reaction vessel, not occupied by the plant material, was estimated by admitting nitrogen gas from the reservoir and noting the change of pressure in the reservoir. The volume of nitrogen taken into the reaction vessel was calculated from the change in pressure. Subtraction of the amount of nitrogen aborbed by the water of the leaves (assuming the absorption to be the same as for pure water) gave the free space in the reaction vessel. The nitrogen was then pumped from the reaction vessel into receiver *h*, transferred to buret *g*, measured, and analyzed for carbon dioxide. The volume of nitrogen obtained in this way usually agreed with the volume absorbed (estimated manometrically) within $\pm$ 0.2 ml.

The absorption of carbon dioxide was then measured in exactly the same manner. The absorption by living leaves was very rapid, ten minutes being sufficient for saturation. In contrast to this, complete absorption by killed leaves required several hours.

By making a series of measurements at increasing pressures of carbon dioxide, the relation between the absorption of carbon dioxide and its partial pressure was obtained.

When the absorptions were completed, the gas was collected in receptacle *h* and the carbon dioxide determined. The agreement between the volumes of gas measured manometrically and volumetrically demonstrated the reversibility of the absorption process.

Diffusion of the water vapor from the reaction vessel through the tube J into the reservoir G was prevented by keeping stopcock K closed most of

the time. It was opened frequently for very short intervals to establish equilibrium between the two vessels.

At equilibrium the partial pressure of the gas being absorbed was equal to the gas pressure in reservoir G less the vapor pressure of the material in the reaction flask. With leaves in the reaction vessel, this vapor pressure was taken as the vapor pressure of pure water (12.8 mm. at 15.0° C.).

When the carbon dioxide was to be liberated from leaves by the addition of acid the reaction vessel L′ was always used. In these instances the absorption measurements were carried out as has been described, the carbon dioxide was pumped off as completely as possible, and 10 ml. of 6 N hydrochloric acid containing one drop of heptyl alcohol was added from the addition funnel. The gas which was liberated was collected in the receiver *h* and the total volume of carbon dioxide determined. During the evolution of the gas the reaction vessel was shaken continuously.

After an experiment had been completed the material was removed from the reaction flask and dried in an oven at 110° C. The water content thus obtained was used to estimate the amount of carbon dioxide dissolved by the water in the absorbing system, on the assumption that the water of the leaf absorbed the same quantity of carbon dioxide as pure water.

GAS-FLOW METHOD.—In a number of experiments (tables I and II) another type of absorption method was used. Instead of using pure carbon dioxide gas at various pressures, the absorptions were measured at various

TABLE I

ABSORPTION OF CARBON DIOXIDE BY LIVING LEAVES FROM DIFFERENT SPECIES OF PLANTS. FRESH WEIGHT, 10.00 GRAMS; TEMPERATURE, 15.0 ± 0.2° C.

| SAMPLE NUMBER | LEAF MATERIAL | $CO_2$ IN GAS | $CO_2$ ABSORBED | $CO_2$ CALC. FOR WATER | $CO_2$ EXCESS |
|---|---|---|---|---|---|
| | | % | *ml.* | *ml.* | *ml.* |
| 1 | *Helianthus annuus* | 98.5 | 10.20 | 8.01 | 2.19 |
| 2 | *Malva parviflora* | 100.0 | 11.93 | 7.77 | 4.16 |
| 3 | *Libo cedrus* | 99.7 | 7.66 | 5.01 | 2.65 |
| 4 | *Eschscholtzia californica* | 98.9 | 9.03 | 7.61 | 1.42 |
| 5 | *Vicia sativa* | 99.7 | 10.01 | 7.38 | 2.63 |
| 6 | *Trifolium repens* | 98.5 | 10.17 | 7.64 | 2.53 |
| 7 | *Quercus douglasii* | 99.7 | 6.38 | 5.56 | 0.82 |
| 8 | *Nicotiana tabacum* | 98.8 | 9.14 | 8.03 | 1.11 |
| 9 | *Berberis aquifolium* (young leaves) | 100.0 | 8.39 | 7.62 | 0.77 |
| 10 | *Berberis aquifolium* (old leaves) | 99.7 | 6.68 | 4.86 | 1.82 |
| 11 | *Polypodium vulgare* | 98.8 | 8.53 | 7.96 | 0.57 |
| 12 | *Prunus cerasifera* var. *pissardii* | 99.2 | 7.86 | 6.46 | 1.40 |
| 13 | *Hordeum vulgare* | 98.5 | 9.61 | 7.75 | 1.86 |
| 14 | *Rosa* sp. | 98.5 | 8.03 | 5.92 | 2.11 |
| 15 | *Helianthus annuus* (normal) | 98.2 | 10.40 | 7.46 | 2.94 |
| 16 | *Helianthus annuus* (starved) | 98.2 | 11.55 | 7.89 | 3.66 |

TABLE II

ABSORPTION OF CARBON DIOXIDE BY VARIOUS PLANT ORGANS. FRESH WEIGHT, 10.00 GRAMS; TEMPERATURE, 15.0 ± 0.2° C.

| SAMPLE NUMBER | PLANT ORGAN | PLANT SPECIES | $CO_2$ IN GAS | $CO_2$ ABS. | $CO_2$ CALC. FOR WATER | $CO_2$ EXCESS |
|---|---|---|---|---|---|---|
| | | | % | *ml.* | *ml.* | *ml.* |
| 1 | Petals | *Eschscholtzia californica* | 99.6 | 8.23 | 8.24 | −0.01 |
| 2 | " | *Rosa* sp. | 99.2 | 9.06 | 8.22 | 0.84 |
| 3 | Coleoptile | *Hordeum vulgare* | 99.7 | 9.72 | 9.00 | 0.72 |
| 4 | Roots | " " | 98.2 | 9.63 | 8.58 | 1.05 |
| 5 | " | " " | 98.8 | 9.30 | 8.30 | 1.00 |
| 6 | Leaves (etiolated) | " " | 98.8 | 9.90 | 8.60 | 1.29 |

partial pressures of carbon dioxide in nitrogen. The saturation was carried out by placing the leaves in reaction chamber G (fig. 2), and passing the

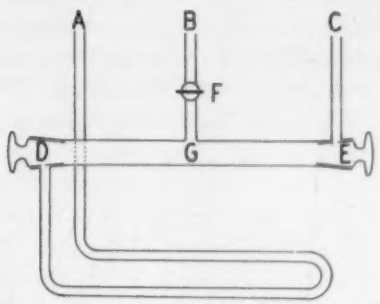



FIG. 2. Vessel for saturating leaf material with carbon dioxide at different partial pressures in a gas stream.

desired gas mixture through the chamber. The chamber G was connected through ground joints to the Sprengel pump at B and the gas supply at A.

Before saturation was begun, the chamber was closed off from tubes A and C by means of the perforated stoppers D and E, then evacuated by the Sprengel pump through the stopcock F. The reaction vessel was shut off from the Sprengel pump and gas was admitted to the chamber through the stopper D and allowed to flow through the chamber by opening stopper E. The end stoppers were then closed and the gas from the reaction vessel pumped into receiver *h*. The volume of the gas was measured in buret *g*. This volume minus the free space (obtained by a similar manipulation with nitrogen) gave the volume of carbon dioxide absorbed. The percentage of carbon dioxide in the gas phase at equilibrium was calculated from the expression

$$\frac{V_r - V_a}{V_f} \times 100$$

where $V_r$ is the volume of carbon dioxide in the gas pumped from the reaction vessel; $V_a$ is the volume of gas absorbed; and $V_f$ is the volume of the free space in the reaction vessel. $V_a = V_t - V_f$, where $V_t$ is the total volume pumped out.

The carbon dioxide absorbed in excess of that attributable to the water was calculated from the equation, $V_e = V_a - V_w$. $V_w$ is the product of the weight of water present in the leaves (grams) times the absorption coefficient of carbon dioxide in pure water. The values of $V_e$ are shown in the last columns of tables I and II.

PREPARATION OF LEAF MATERIALS FOR ABSORPTION.—The fresh leaves after being harvested were washed with distilled water to clean off the surface dirt. The water remaining on the surfaces of the smaller leaves was removed by absorption in filter paper. Water was removed from the surfaces of the larger leaves by evaporation; the petioles were immersed in distilled water during this process.

The parenchymatous tissue of the larger leaves was removed from the main veins and cut into pieces approximately one square inch in area. Smaller leaves were used whole.

This material was weighed into the reaction flask and the carbon dioxide absorption measured. Ten grams ($\pm$ 0.02 gm.) of material was used in all but a few cases where this amount of material was not available.

METHODS USED FOR KILLING LEAVES.—Leaves to be killed by freezing were harvested and washed in the manner already described. A 10-gm. sample was weighed into a small capped vial and immersed to the cap in a freezing bath of ethyl acetate and solid carbon dioxide. When the leaves had become frozen the tubes were removed and the leaf material allowed to thaw. This material was weighed into the reaction flask and used for absorption experiments.

The amount of water lost from the leaves during these manipulations was only about 1 per cent. Duplicate samples of leaves, one fresh, the other frozen, gave 14.9 and 15.5 per cent. dry weight, respectively.

Leaves to be killed by heating were placed into small capped vials and immersed in boiling water for from twenty to thirty minutes. The vials were allowed to cool before the contents were removed.

## ABSORPTION OF CARBON DIOXIDE BY UNILLUMINATED LEAVES

Absorption of carbon dioxide by unilluminated leaves in excess of that ascribable to the water present is a property that is widespread among a number of species of plants (tables I and II). Only in one species so far examined was the amount of carbon dioxide *absorbed* less than that calcu-

lated for the water present and even in this case the volume of carbon dioxide *removed* by pumping was greater than that reckoned for the water. This plant, *Sedum praealtum*, has an acid sap, pH 4.08 (table III, no. 26).

Roots and coleoptiles of barley plants and the petals of roses also absorbed carbon dioxide in excess of the amount attributable to water. Petals from the California poppy (*Eschscholtzia californica*) were an exception (table II). At low pressures of carbon dioxide the absorption by these organs was more nearly proportional to the pressure than was the absorption of carbon dioxide by leaves.[1]

Starvation of leaves affected their ability to absorb carbon dioxide. Sunflower leaves which had been in sunlight all day and contained an abundant supply of starch (table I, no. 15) absorbed less carbon dioxide than similar leaves, cut from the same plants, which had been stored in the dark over night and had lost their starch (table I, no. 16). Leaves which lacked green pigments, however, absorbed carbon dioxide as shown in table III.

### The reversibility of the carbon dioxide absorption

Carbon dioxide, to become available for photosynthesis, may have to dissociate from the substances by which it is first absorbed. This reversibility would then be an important property of the absorption system. For this reason the reversibility of the absorption process was measured for leaves of different species of plants and for different kinds of leaves from the same species.

The experiments were carried out in the apparatus shown in figure 1. The amount of gas absorbed, measured manometrically, was compared with the amount of gas that could be removed by pumping (table III, columns 6 to 8). Once the leaves had been treated with carbon dioxide the amounts of gas absorbed and removed were more nearly equal in subsequent treatments. This is shown by comparing the experiments lettered (a) with those lettered (b) and (c) in table III.

Complete reversibility was demonstrated for all the leaves examined, whether they were green, variegated yellow (no. 25), albino (no. 4), or etiolated (nos. 2, 9, 11, 16, 23). It is apparent from these results that the green pigment is not the principal factor involved in the reversible absorption of carbon dioxide by leaves. This reversibility is characteristic of killed leaves (table III, nos. 27–30) as well as of living leaves, consequently it is not dependent on some life process in the leaves.

In most instances the amount of carbon dioxide removed from living leaves was greater than that absorbed. This additional carbon dioxide undoubtedly came from the respiration of the leaves. Its production not only increased the amount of carbon dioxide removable from the leaves but de-

[1] The results recorded in tables I and II were obtained by means of the gas-flow method, (*cf.* fig. 2); those in table III, by apparatus, figure 1.

## TABLE III

REVERSIBILITY OF CARBON DIOXIDE ABSORPTION BY LEAVES

| SAMPLE NUMBER | LEAF MATERIAL | FRESH WT. | DRY WT. | PRESS. $CO_2$ AT EQUIL. | VOL. $CO_2$ ABSORB. | VOL. $CO_2$ REMOVED | $\frac{CO_2 \text{ REM.}}{CO_2 \text{ ABS.}}$ | EXCESS $CO_2$ ABS. | EXCESS $CO_2$ REM. | EXCESS $CO_2$ ABS. AT 1 ATMOS. |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Zea mays** | *gm.* | *gm.* | *mm.* | *ml.* | *ml.* | | *ml.* | *ml.* | *ml.* |
| 1 | a Green | 10.00 | 1.18 | 645.8 | 8.73 | 9.09 | 1.041 | 1.08 | 1.44 | 1.19 |
| | b " | 10.00 | 1.18 | 515.5 | 7.22 | 7.48 | 1.036 | 1.11 | 1.37 | |
| 2 | a Etiolated | 10.00 | 0.75 | 645.4 | 8.78 | 9.07 | 1.033 | 0.76 | 1.05 | 0.79 |
| | b " | 10.00 | 0.75 | 508.2 | 7.00 | 7.17 | 1.024 | 0.68 | 0.85 | |
| 3 | a Green | 10.00 | 1.18 | 632.1 | 8.39 | 8.75 | 1.043 | 0.90 | 1.26 | 1.05 |
| | b " | 10.00 | 1.18 | 517.4 | 7.10 | 7.26 | 1.023 | 0.97 | 1.13 | |
| 4 | a Albino | 10.00 | 0.86 | 681.5 | 9.68 | 9.94 | 1.027 | 1.32 | 1.58 | 1.38 |
| | b " | 10.00 | 0.86 | 517.9 | 7.80 | 7.72 | 0.990 | 1.45 | 1.37 | |
| | c " | 10.00 | 0.86 | 517.3 | 7.88 | 7.97 | 1.011 | 1.54 | 1.63 | |
| 5 | a Green (water-rich) | 10.00 | 1.16 | 653.8 | 9.03 | 9.18 | 1.017 | 1.27 | 1.42 | 1.38 |
| | b " " | 10.00 | 1.16 | 512.3 | 7.31 | 7.26 | 0.993 | 1.23 | 1.18 | |
| 6 | a Green (water-poor) | 4.96 | 1.15 | 627.1 | 4.33 | 4.33 | 1.000 | 1.12 | 1.12 | 1.32 |
| | b " " | 4.96 | 1.15 | 515.0 | 3.80 | 3.64 | 0.958 | 1.17 | 1.01 | |
| | *Phaseolus multiflorus* | | | | | | | | | |
| 7 | a Green (water-rich) | 10.00 | 1.40 | 708.4 | 9.42 | 9.80 | 1.040 | 1.25 | 1.63 | 1.29 |
| | b " " | 10.00 | 1.40 | 517.0 | 7.31 | 7.60 | 1.040 | 1.34 | 1.63 | |
| 8 | a Green | 4.49 | 1.32 | 678.3 | 3.61 | 3.82 | 1.058 | 0.73 | 0.94 | 0.77 |
| 9 | a Etiolated | 8.37 | 1.01 | 650.5 | 8.69 | 9.08 | 1.046 | 2.55 | 2.92 | 2.68 |
| | b " | 8.37 | 1.01 | 509.4 | 7.59 | 7.50 | 0.988 | 1.99 | 1.90 | |
| | *Pisum sativum* | | | | | | | | | |
| 10 | a Green | 10.00 | 1.17 | 650.7 | 9.01 | 9.99 | 1.111 | 1.30 | 2.28 | 1.43 |
| | b " | 10.00 | 1.17 | 510.2 | 8.27 | 8.37 | 1.012 | 2.19 | 2.29 | |
| 11 | a Etiolated | 10.00 | 1.13 | 658.8 | 9.50 | 10.29 | 1.083 | 3.49 | 4.28 | 3.66 |
| | b " | 10.00 | 1.13 | 485.9 | 8.39 | 8.52 | 1.015 | 4.20 | 4.33 | |

TABLE III—(*Continued*)

| Sample number | Leaf material | Fresh wt. | Dry wt. | Press. $CO_2$ at equil. | Vol. $CO_2$ absorb. | Vol. $CO_2$ removed | $\frac{CO_2 \text{ rem.}}{CO_2 \text{ abs.}}$ | Excess $CO_2$ abs. | Excess $CO_2$ rem. | Excess $CO_2$ abs. at 1 atmos. |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Helianthus annuus* | *gm.* | *gm.* | *mm.* | *ml.* | *ml.* | | *ml.* | *ml.* | *ml.* |
| 12 | a Green (water-rich) | 10.00 | 1.89 | 656.4 | 9.51 | 9.38 | 0.986 | 2.37 | 2.24 | 2.68 |
| | b " " | 10.00 | 1.89 | 512.1 | 7.79 | 8.10 | 1.040 | 2.22 | 2.53 | |
| 13 | a Green (water-poor) | 5.88 | 1.94 | 652.6 | 4.94 | 4.62 | 0.935 | 1.49 | 1.17 | 1.73 |
| | b " " | 5.88 | 1.94 | 453.3 | 3.65 | 3.59 | 0.984 | 1.25 | 1.19 | |
| | *Nicotiana tabacum* | | | | | | | | | |
| 14 | a Green | 10.00 | 3.26 | 650.1 | 6.85 | 6.75 | 0.985 | 0.97 | 0.87 | 1.03 |
| 15 | a Yellow with age | 10.00 | 0.81 | 705.9 | 8.73 | 8.71 | 0.998 | 0.03 | 0.01 | 0.08 |
| 16 | a Etiolated | 4.70 | 0.40 | 644.1 | 3.88 | 4.14 | 1.067 | 0.34 | 0.60 | 0.34 |
| | *Malva parviflora* | | | | | | | | | |
| 17 | a Green (water-rich) | 10.00 | 1.93 | 661.7 | 10.18 | 11.18 | 1.098 | 3.01 | 4.01 | 3.17 |
| | b " " | 10.00 | 1.93 | 314.1 | 6.20 | 6.58 | 1.061 | 2.80 | 3.18 | |
| | c " " | 10.00 | 1.93 | 503.5 | 8.77 | 9.20 | 1.049 | 3.32 | 3.75 | |
| 18 | a Green (water-poor) | 5.04 | 1.92 | 648.0 | 4.53 | 5.25 | 1.159 | 1.82 | 2.54 | 2.04 |
| | b " " | 5.04 | 1.92 | 277.7 | 2.79 | 2.91 | 1.043 | 1.63 | 1.75 | |
| | c " " | 5.04 | 1.92 | 507.8 | 4.26 | 4.43 | 1.040 | 2.14 | 2.31 | |
| | *Beta vulgaris* | | | | | | | | | |
| 19 | a Green (water-rich) | 10.00 | 1.62 | 642.0 | 7.59 | 7.84 | 1.033 | 0.37 | 0.62 | 0.43 |
| | b " " | 10.00 | 1.62 | 231.3 | 2.96 | 3.09 | 1.044 | 0.36 | 0.49 | |
| | c " " | 10.00 | 1.62 | 513.5 | 6.28 | 6.27 | 0.998 | 0.50 | 0.49 | |
| 20 | a Green (water-poor) | 6.29 | 1.59 | 651.6 | 4.43 | 4 29 | 0.968 | 0.32 | 0.18 | 0.36 |
| | b " " | 6.29 | 1.59 | 299.3 | 2.24 | 2.16 | 0.964 | 0.35 | 0.27 | |
| | c " " | 6.29 | 1.59 | 519.3 | 3.52 | 3.50 | 0.994 | 0.25 | 0.23 | |

TABLE III—(*Concluded*)

| Sample number | Leaf material | Fresh wt. | Dry wt. | Press. $CO_2$ at equil. | Vol. $CO_2$ absorb. | Vol. $CO_2$ removed | $\frac{CO_2 \text{ rem.}}{CO_2 \text{ abs.}}$ | Excess $CO_2$ abs. | Excess $CO_2$ rem. | Excess $CO_2$ abs. at 1 atmos. |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Hordeum vulgare* | *gm.* | *gm.* | *mm.* | *ml.* | *ml.* | | *ml.* | *ml.* | *ml.* |
| 21 | a Green (water-rich) | 10.00 | 0.94 | 647.1 | 8.57 | 9.64 | 1.125 | 0.70 | 1.77 | 0.75 |
| | b " " | 10.00 | 0.94 | 248.9 | 3.89 | 4.45 | 1.144 | 0.86 | 1.42 | |
| | c " " | 10.00 | 0.94 | 516.1 | 7.73 | 8.04 | 1.040 | 1.46 | 1.77 | |
| 22 | a Green (water-poor) | 6.74 | 0.91 | 662.1 | 6.10 | 6.63 | 1.087 | 0.92 | 1.45 | 0.93 |
| | b " " | 6.74 | 0.91 | 230.1 | 2.74 | 2.81 | 1.026 | 0.94 | 1.01 | |
| | c " " | 6.74 | 0.91 | 504.8 | 5.31 | 5.65 | 1.064 | 1.36 | 1.70 | |
| 23 | a Etiolated | 10.00 | 0.79 | 619.2 | 8.26 | 9.38 | 1.136 | 0.61 | 1.73 | 0.62 |
| | b " | 10.00 | 0.79 | 157.7 | 2.86 | 3.07 | 1.074 | 0.91 | 1.12 | |
| | c " | 10.00 | 0.79 | 490.3 | 7.46 | 7.46 | 1.000 | 1.40 | 1.40 | |
| | *Evonymus japonica* | | | | | | | | | |
| 24 | a Green | 10.00 | 4.50 | 664.1 | 5.73 | 7.58 | 1.323 | 0.83 | 2.68 | 0.86 |
| 25 | a Yellow | 10.00 | 2.54 | 668.6 | 8.11 | 9.30 | 1.147 | 1.42 | 2.61 | 1.54 |
| | *Sedum praealtum* | | | | | | | | | |
| 26 | a | 10.00 | 0.74 | 645.2 | 7.31 | 8.19 | 1.120 | −0.72 | 0.16 | −0.79† |
| | b " " | 10.00 | 0.74 | 235.6 | 2.69 | 3.36 | 1.249 | −0.24 | 0.43 | |
| | c " " | 10.00 | 0.74 | 505.9 | 6.05 | 6.58 | 1.088 | −0.24 | 0.29 | |
| | *Helianthus annuus* | | | | | | | | | |
| 27 | a Green (heated) | 9.41 | 1.58 | 574.3 | 16.36 | 16.05 | 0.981 | 10.32 | 10.11 | 12.20 |
| 28 | a Green (frozen) | 9.84 | 1.74 | 663.6 | 21.20 | 20.54 | 0.970 | 13.99 | 13.33 | 14.43 |
| 29 | a Green (ext. with $H_2O$) | | 1.33 | 670.1 | 16.48 | 16.47 | 0.999 | 4.15 | 4.14 | 4.20 |
| 30 | a Green (ext. $CO_2$–$H_2O$) | | 1.24 | 679.5 | 11.59 | 11.42 | 0.985 | 0.11 | −0.06 | 0.15 |

* Corn seedlings nos. 1 and 2 (variety Golden Bantam) were grown from the same lot of seed.

The corn seedlings (nos. 3, 4, 5 and 6) were grown from seed furnished by PROFESSOR A. C. FRAZER, Cornell University. Samples 5 and 6 were from the same lot of seed and grown under comparable conditions. The tobacco leaves (no. 16) were grown on fully developed tobacco plants which had been kept in the dark for some weeks. † An excess of 0.13 instead of a deficit of −0.79 is obtained when the solubility coefficient for the water of the sap is substituted for that of pure water (*cf.* p. 200).

creased the absorption of an equivalent amount of carbon dioxide from the gas reservoir. This made the amount of gas absorbed too small (table III, column 9). For this reason it may be that the amount of gas removed by pumping (table III, column 10) is a better measure of the absorption than the gas uptake, measured manometrically. The amount of carbon dioxide removed from killed leaves was usually slightly less than the amount taken up. This deficit was reduced to an insignificant amount if the period of pumping was greatly prolonged; hours of pumping were often required to remove the last noticeable quantity of gas. Perhaps the cause of this was the slow diffusion of gas in the killed leaf.

One conspicuous difference observed between living and killed leaves (killed by either heating or freezing) was the rate at which each attained equilibrium with its surrounding atmosphere. Living leaves absorbed or evolved carbon dioxide very quickly and reached equilibrium within a very few minutes. Killed leaves, however, required hours to complete the reaction. This retardation in reactivity is probably caused by the breaking down of the structure of the leaf, which would make diffusion much slower. The retardation also might be caused by the inhibition of the chemical reactions with carbon dioxide.

Summarizing, it may be said that the uptake of carbon dioxide by the unilluminated leaf is strictly a reversible reaction which is independent of the presence of chlorophyll and of the life processes of the leaf.

### Absorption of carbon dioxide by different extracts from sunflower leaves

Preliminary to an investigation of what constituents in the leaf combine with carbon dioxide, the absorption and evolution (by acidification) of carbon dioxide by different extracts from sunflower leaves were determined. Frozen sunflower leaves were extracted with water and with water saturated with carbon dioxide. Sunflower leaves were chosen because of their large carbon dioxide absorption capacity, which has been remarked by others (**33b**, **28**). In order to obtain the extracts the leaves had to be killed. For this reason it was desirable to determine the effect of killing.

Examination showed that the leaves killed by freezing ($-70°$) absorbed reversibly considerably more carbon dioxide (exclusive of that dissolved by the water of the leaf) than did living leaves (reversible $CO_2$, table IV, nos. 1 and 2). On the other hand, killed leaves evolved less carbon dioxide when treated with cold dilute hydrochloric acid (irreversible $CO_2$). When saturated with carbon dioxide at one atmosphere of pressure, the living and killed leaves contained approximately the same quantity of carbon dioxide (total combined $CO_2$).

The frozen sunflower leaves were extracted with water. Both the soluble constituents (no. 3) and the insoluble leaf residue (no. 4) absorbed

carbon dioxide (table IV, reversible $CO_2$), but only the insoluble residue liberated carbon dioxide on treatment with acid (nos. 3 and 4, irreversible $CO_2$). The total combined $CO_2$ in the residue was nearly double that in the dissolved material.

## TABLE IV

THE DISTRIBUTION OF THE CARBON DIOXIDE COMBINED BY FRACTIONS OF SUNFLOWER LEAVES, SEPARATED BY DIFFERENCE IN SOLUBILITY. ALL VALUES RECORDED ARE BASED ON 10.00 GRAMS FRESH WEIGHT OF LEAVES. THE LEAVES USED IN NOS. 1 TO 6 WERE ALIQUOT PORTIONS TAKEN FROM THE SAME LOT OF LEAVES

| SAMPLE NUMBER | LEAF MATERIAL | CARBON DIOXIDE COMBINED* | | |
|---|---|---|---|---|
| | | REVERSIBLE $CO_2$ | IRREVERSIBLE $CO_2$ | TOTAL COMBINED $CO_2$ |
| | | *ml.* | *ml.* | *ml.* |
| 1 | Living leaf | 2.98 | 15.78 | 18.76 |
| 2 | Frozen leaf | 8.89 | 7.83 | 16.72 |
| 3 | Water-soluble | 4.82 | − 0.31 | 4.51 |
| 4 | Water-insoluble | 4.88 | 4.28 | 9.16 |
| 5 | $CO_2$-water-soluble | 5.94 | 5.30 | 11.24 |
| 6 | $CO_2$-water-insoluble | 0.00 | 0.46 | 0.46 |
| 7 | Leaf sap | 2.42 | (8.84) † | |

* Reversibly combined $CO_2$. The amount of carbon dioxide absorbed by the leaf material, at a partial pressure of carbon dioxide equal to 1 atmosphere, in excess of the amount calculated to saturate the water present.

Irreversibly-combined $CO_2$. The amount of carbon dioxide that is liberated from the evacuated leaf material on treatment with cold dilute acid. In practice it is the amount of carbon dioxide obtained from the material (in equilibrium with a given partial pressure of carbon dioxide) when acidified, minus the amount of carbon dioxide absorbed by the leaf when the pressure of carbon dioxide is changed from zero to the equilibrium pressure.

Total combined $CO_2$. The sum of the reversible and irreversible carbon dioxide.

† This result was determined with the Van Slyke blood-gas analysis apparatus instead of the apparatus shown in figure 1 which was used for the other measurements.

The water-soluble substances were removed from the leaves (10.00 gm.) killed by freezing in the following manner. The sample of killed leaves was extracted thoroughly with three 100-ml. portions of distilled water. The solid residue was collected, rinsed with distilled water, transferred to the reaction flask, and its absorption measured (table IV, no. 4).

The extract was evaporated to dryness in a platinum dish on the water bath. The solid remaining was transferred quantitatively to the reaction flask by the use of distilled water and the absorption and evolution of carbon dioxide by this material measured (table IV, no. 3).

Although the water-soluble material failed to evolve carbon dioxide when treated with dilute hydrochloric acid, other experiments demonstrated that the leaf contains water-soluble substances which liberate carbon dioxide on acidification. It is probable, therefore, that these substances were decomposed with loss of carbon dioxide during the evaporation process.

The leaf residue, which was insoluble in water, was further extracted

with water saturated with carbon dioxide. The residue insoluble in this reagent (no. 6) did not absorb carbon dioxide nor did it liberate carbon dioxide when treated with cold dilute hydrochloric acid. The soluble material (no. 5), however, absorbed carbon dioxide and also liberated carbon dioxide when treated with acid. These observations indicated that the substances insoluble in water which are responsible for these results are carbonates.

A graphical summary is here given of the distribution of the volumes of carbon dioxide combined by the leaf constituents in the different fractions obtained by extraction:

Frozen leaf 16.72 ml.—
- Water-soluble 4.51 ml.
- Water-insoluble 9.16 ml.—
  - Water-$CO_2$-soluble 11.24 ml.
  - Water-$CO_2$-insoluble 0.46 ml.

The sap expressed from frozen sunflower leaves (no. 7) was shown to contain substances which absorb carbon dioxide, and also to liberate carbon dioxide when treated with acid (this sample of sap was not expressed from leaves taken from the same lot as those used in experiments 1 to 6).

These experiments clearly demonstrate that sunflower leaves possess a carbon-dioxide-absorption system which is divided between the sap and the solid leaf material. This system provides a reservoir of carbon dioxide within the leaf which may be available for photosynthesis.

The notable differences between living and killed leaves in the amounts of reversible and irreversible carbon dioxide contained by each (table IV, nos. 1 and 2) may possibly be explained by the inequality in their rates of respiration.

Preliminary to measuring the carbon dioxide absorption the leaves were thoroughly evacuated. This removed all of the dissociable carbon dioxide. Since the living leaves were respiring rapidly, any carbon dioxide absorbents that they contained would be kept saturated. On the other hand, the frozen leaves respired relatively slowly and once these absorbents had been freed of dissociable carbon dioxide they would become saturated again only very slowly. Thus the living leaves would possess a low absorption capacity and a large quantity of irreversible carbon dioxide whereas frozen leaves would have these properties reversed.

Other experiments corroborated this conjecture. Three 10-gm. samples of sunflower leaves were killed in three different ways and the amounts of carbon dioxide that they retained were measured. Leaves killed with ether at room temperature retained 17.30 ml. of carbon dioxide; leaves killed by drying in an atmosphere of carbon dioxide retained 13.42 ml.; leaves killed by heating at the boiling point of water retained only 10.55 ml. These

results are what would be expected if the carbon dioxide of respiration were retained in the leaves by easily dissociable compounds. Leaves killed without heating or drying would retain the most carbon dioxide, while leaves heated without drying would retain the least.[2]

Although the counterpart of the experiment with frozen leaves has not been performed with leaves killed by heating, the latter absorb more carbon dioxide than similar living leaves. Heat-killed leaves retain a considerable quantity of carbon dioxide that is liberated only by treatment with acid.

### Absorption of carbon dioxide by particular leaf constituents

Absorption by water.—In order to determine the influence of water, measurements were made of the carbon dioxide absorption by matched samples of leaves which contained different amounts of water.

These samples were obtained in the following manner. Large leaves were halved and the halves segregated into two portions of 10.00 gm. each. Small leaves were thoroughly mixed and aliquot portions of 10.00 gm. each withdrawn. One portion was used immediately for absorption measurements whereas the other was partially dried before use.

The results of these experiments show that leaves with low water content absorb less carbon dioxide than those with normal water content, (fig. 3). The removal of water from leaves reduced their capacity to

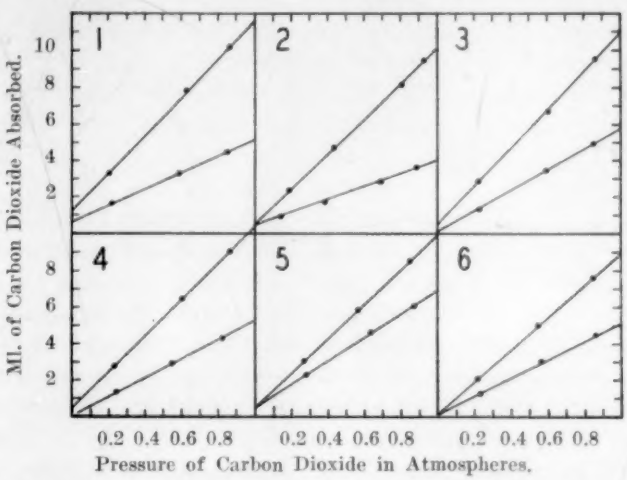



Fig. 3. The influence of water content on the absorption of carbon dioxide by leaves. The upper curves represent absorption by leaves with normal water content; the lower curves, absorption by leaves with diminished water content.

1) *Malva parviflora*; 2) *Phaseolus multiflorus*; 3) *Helianthus annuus*; 4) *Zea mays*; 5) *Hordeum vulgare*; 6) *Beta vulgaris*.

[2] The carbon dioxide retained by these various samples of leaves was estimated by liberating the carbon dioxide with cold dilute hydrochloric acid and absorbing it in a

absorb carbon dioxide to a greater extent than was calculated on the basis of water loss. This is what would be expected if the water is present in the leaf in a dilute aqueous solution such as a sugar solution. As the solution becomes more concentrated its absorption capacity is decreased both by the loss of water and by the salting-out effect (table V). Only one sample of leaves showed an apparent exception to this (table V, no. 2).

TABLE V

CHANGE IN $CO_2$ ABSORPTION WITH CHANGE IN WATER CONTENT. ORIGINAL FRESH WEIGHT 10.00 GRAMS: TEMPERATURE 15° C.

| SAMPLE NUMBER | LEAF MATERIAL | WATER CONTENT | SOLUBILITY AT $P_{CO_2} = 1$ ATMOSPHERE | DECREASE IN WATER CONTENT | DECREASE IN $CO_2$ ABSORPTION | RATIO $\frac{d\,CO_2}{d\,H_2O}$ |
|---|---|---|---|---|---|---|
| | | *gm.* | *ml.* | *gm.* | *ml.* | |
| 1 | *Zea mays* | 8.84 | 10.43 | | | |
| | (seedlings) | 3.81 | 5.22 | 5.03 | 5.21 | 1.036 |
| 2 | *Hordeum vulgare* | 9.06 | 9.98 | | | |
| | | 5.83 | 6.92 | 3.23 | 3.06 | 0.947 |
| 3 | *Beta vulgaris* | 8.38 | 8.96 | | | |
| | | 4.70 | 5.14 | 3.68 | 3.82 | 1.038 |
| 4 | *Malva parviflora* | 8.07 | 11.51 | | | |
| | | 3.12 | 5.21 | 4.95 | 6.30 | 1.273 |
| 5 | *Phaseolus multiflorus* | 8.60 | 10.06 | | | |
| | | 3.17 | 3.99 | 5.43 | 6.07 | 1.118 |
| 6 | *Helianthus annuus* | 8.11 | 10.93 | | | |
| | | 3.94 | 5.74 | 4.17 | 5.19 | 1.245 |
| 7 | Water | | | | | 1.020 |
| 8 | Sugar solution (20°)* | 972.4 | 846.0 | | | |
| | | 786.1 | 649.0 | 186.3 | 197.0 | 1.058 |
| 9 | Water (20°) | | | | | 0.880 |

* QUINN, ELTON L., and JONES, CHARLES L., Carbon dioxide, pp. 103, Reinhold Publishing Corporation. 1936.

Within the pressure range, 0.3 to 1.0 atmosphere, the solubility of carbon dioxide increased proportionally to the increase in pressure. If the increase in solubility per atmosphere of pressure is divided by the number of grams of water contained in the leaves, an apparent solubility coefficient is obtained for the water in the leaf (table VI, column 6).

The apparent solubility coefficient, in most cases, is greater than the coefficient for pure water. The coefficient increases as the water content decreases. Therefore there are substances in the leaf besides water which absorb carbon dioxide in proportion to its pressure. These substances are not lost by the partial dehydration of the leaf but increase in amount rela-

known quantity of standard barium hydroxide and determining the excess barium hydroxide with standard hydrochloric acid.

## TABLE VI

CORRELATION OF THE CARBON DIOXIDE ABSORPTION COEFFICIENT OF LEAVES WITH THEIR WATER CONTENT. ORIGINAL WEIGHT OF LEAF MATERIAL, 10.00 GRAMS; TEMPERATURE 15° C.

| SAMPLE NUMBER | LEAF MATERIAL | WATER CONTENT | $d V_{CO_2}$ | $dP_{CO_2}$ | $\frac{d V_{CO_2}}{d P_{CO_2} W_{H_2O}}$ |
|---|---|---|---|---|---|
| | | *gm.* | *ml.* | *atm.* | |
| 1 | *Zea mays* | 8.84 | 6.23 | 0.6233 | 1.131 |
| | | 3.81 | 2.98 | 0.5842 | 1.339 |
| 2 | *Hordeum vulgare* | 9.06 | 5.51 | 0.5791 | 1.050 |
| | | 5.83 | 3.77 | 0.5911 | 1.094 |
| 3 | *Beta vulgaris* | 8.38 | 5.57 | 0.6330 | 1.050 |
| | | 4.70 | 3.13 | 0.6274 | 1.061 |
| 4 | *Malva parviflora* | 8.07 | 6.89 | 0.6686 | 1.277 |
| | | 3.12 | 2.92 | 0.6354 | 1.473 |
| 5 | *Phascolus multiflorus* | 8.60 | 7.05 | 0.7434 | 1.103 |
| | | 3.17 | 2.66 | 0.7483 | 1.121 |
| 6 | *Helianthus annuus* | 8.11 | 6.65 | 0.6404 | 1.280 |
| | | 3.94 | 3.59 | 0.6320 | 1.442 |
| 7 | *Sedum praealtum* | 9.26 | 4.79 | 0.5781 | 0.895 |
| 8 | Water | | | | 1.020 |

tive to the water remaining. If such substances were not present the apparent solubility coefficient for carbon dioxide would be less than for pure water, due to the presence of neutral substances dissolved in the sap. In fact, leaf saps acidified in order to prevent neutralization of carbonic acid by basic constituents contained therein, possess solubility coefficients considerably less than pure water. For example, at 15° C., the solubility coefficients [ml. $CO_2$ (0°, 760 mm.)/gm. $H_2O$/atm. $CO_2$] for two saps acidified to pH 3.2 are: *Helianthus annuus*, 0.926; *Sedum praealtum*, 0.913. Hence it is not surprising that leaves of *Sedum praealtum* which contain an acid sap (pH 4.08) had an apparent solubility coefficient less than water (table VI, no. 7).

INFLUENCE OF HYDROGEN-ION CONCENTRATION.—Since aqueous solutions of carbon dioxide are acid, it is probable that the hydrogen-ion concentrations of leaf saps might control the ability of leaves to absorb carbon dioxide. It is obvious that in leaf saps the amount of hydroxyl ion available to react directly with carbon dioxide is too small to be of significance. The concentration of hydroxyl ion, however, may influence secondary reactions such as the formation of the alkaline-earth carbonates which are important in the process of carbon dioxide absorption. For this reason a statistical survey was made of the relation between the pH of leaf saps (obtained from heated leaves) and the amount of carbon dioxide absorbed by these leaves (corrected for the carbon dioxide dissolved by the water) at one atmosphere

pressure of carbon dioxide. The results show that little if any correlation exists between the pH of the saps and their carbon dioxide absorption (fig. 4).

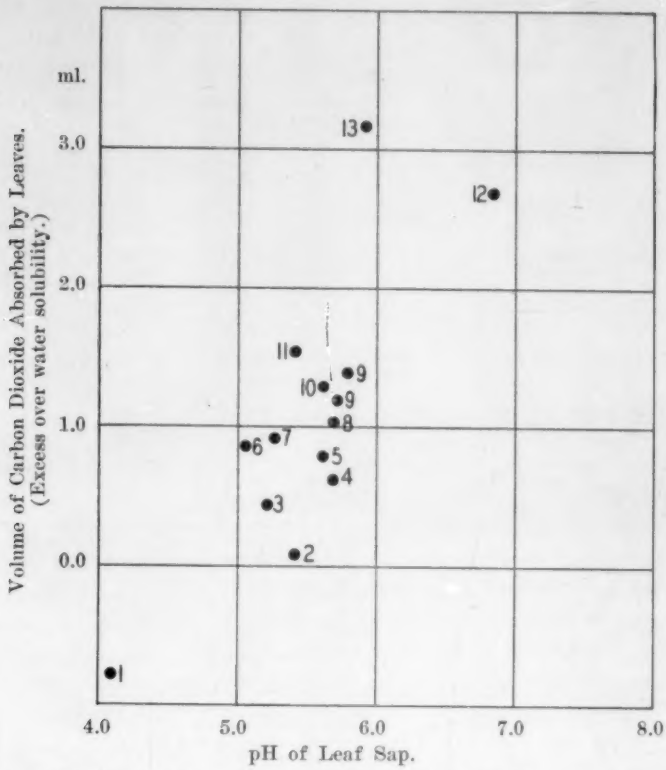



FIG. 4. Influence of hydrogen ion activity on the absorption of carbon dioxide by different leaves.

Ordinate: ml. of carbon dioxide absorbed in excess of that attributable to the water. Abscissa: pH of the saps expressed from heat-killed leaves.

1) *Sedum praealtum*; 2) *Nicotiana tabacum*, yellow with age; 3) *Beta vulgaris*; 4) *Hordeum vulgare*, etiolated; 5) *Zea mays*, etiolated; 6) *Evonymus japonicus*; 7) *Hordeum vulgare*; 8) *Nicotiana tabacum*; 9) *Zea mays*; 10) *Phaseolus multiflorus*; 11) *Evonymus japonicus*, yellow; 12) *Helianthus annuus*; 13) *Malva parviflora*.

When not otherwise specified, green leaves were used.

ABSORPTION BY LEAF-SAP CONSTITUENTS.—The absorption of carbon dioxide by the sap from frozen sunflower leaves was analyzed in order to determine what type of absorption takes place. For example, does the carbon dioxide add directly to the sap constituents, as in carbamino-acid formation, or is it neutralized to form bicarbonate ion? To differentiate between these two modes of absorption the quantity of carbon dioxide that was chemically

bound in the sap was determined and compared with the amount of bicarbonate ion formed.

PRIMARY IONIZATION CONSTANT OF CARBONIC ACID.—The most convenient way to obtain the bicarbonate ion concentration in the leaf sap is to calculate it from the equation defining the ionization constant of carbonic acid,

$$pK = pH + pHCO_3 + pf_{HCO_3} - pP_{CO_2} - pS_{CO_2},^3$$

that is, provided that the value of this constant is known and conditions of measurement can be arranged so as to warrant its use.

To establish that the experimental conditions warranted the use of this equation, the ionization constant was determined under the conditions which were used for the measurements on leaf saps. The values for the individual terms of the equation were obtained in the following ways: The activities of the hydrogen ion in the various solutions, saturated with carbon dioxide in the cell shown in figure 5, were measured by means of a Beckman pH meter.

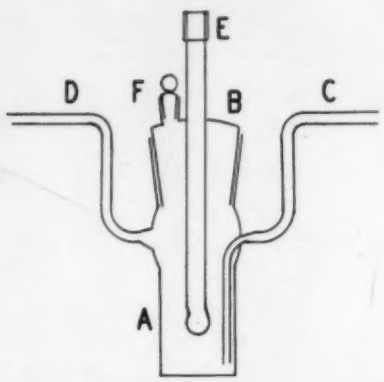



FIG. 5. Gas-reaction cell for pH measurements. A, container; B, ground-glass cap carrying the calomel electrode (not shown) and glass electrode, E; F, opening for removal of samples; C, inlet tube; D, exit for the passage of the gas stream.

The concentrations of the 0.1 M solutions of bicarbonate were determined by acidimetry.[4] The weaker solutions were made by dilution of the 0.1 M solutions. The partial pressures of the carbon dioxide were calculated from the composition of the carbon dioxide-nitrogen mixtures, the barometric pressures, and the vapor pressures of the solutions. The solubility of carbon dioxide, 0.0454 mol/liter/atmosphere, was taken from the literature (**3**).

[3] In this expression p is (− log of a given quantity); K, the primary ionization constant of carbonic acid; H, the hydrogen ion activity; $HCO_3$, the bicarbonate ion concentration; $f_{HCO_3}$, the activity coefficient of the bicarbonate ion; $P_{CO_2}$, the partial pressure of the carbon dioxide in atmospheres; $S_{CO_2}$, the solubility of carbon dioxide in water expressed in mol/liter/atmospheres.

[4] Merck's reagent grade of sodium bicarbonate was used for preparing the bicarbonate solutions.

The activity coefficients of the bicarbonate ion were calculated from the ionic strengths of the solutions according to the formula proposed by GUGGENHEIM and SCHINDLER (**10**).

$$-\log f = 0.5\ Z_i^2 \frac{\sqrt{\mu}}{1+\sqrt{\mu}} + \sum_k B_{ik} C_k;$$

which for the bicarbonate ion in the solutions used becomes

$$pf_{HCO_3} = 0.5 \frac{\sqrt{\mu}}{1+\sqrt{\mu}} + 0.221\ C_{Na^+} + 0.044 C_{K^+}.$$

$C_{Na^+}$ and $C_{K^+}$ are the molar concentrations of the sodium and potassium ions and $\mu$ is the ionic strength in the solutions measured.

In table VII are given the values of pK for the primary ionization constant of carbonic acid. The average value, pK 6.4252, is in good agreement with the value reported by SHEDLOVSKY and MACINNES (**21**), *viz.*, 6.4293 at 15.0° C.

## TABLE VII

DATA FOR CALCULATING THE IONIZATION CONTANT OF CARBONIC ACID

| $NaHCO_3$ MOL/L. | $\sqrt{\mu}$ | $P_{CO_2}$ | $pP_{CO_2}$ | pH | $pHCO_3$ | $pf_{HCO_3}$ | pK |
|---|---|---|---|---|---|---|---|
| 0.0009936 | 0.0315 | 38.04 | 1.301 | 6.050 | 3.003 | 0.015 | 6.424 |
| " | " | 152.7 | 0.699 | 5.450 | " | " | 6.426 |
| " | " | 743.4 | 0.009 | 4.770 | " | " | 6.436 |
| 0.009936 | 0.0996 | 38.01 | 1.301 | 7.020 | 2.003 | 0.047 | 6.426 |
| " | " | 152.5 | 0.698 | 6.415 | " | " | 6.424 |
| " | " | 742.9 | 0.010 | 5.725 | " | " | 6.422 |
| 0.09936 | 0.315 | 37.98 | 1.301 | 7.926 | 1.003 | 0.140 | 6.425 |
| " | " | 152.6 | 0.697 | 7.323 | " | " | 6.426 |
| " | " | 742.9 | 0.010 | 6.640 | " | " | 6.430 |
| | | | | | | AVERAGE | 6.426 |

IN ANOTHER SERIES OF MEASUREMENTS THE $NaHCO_3$ WAS MAINTAINED AT 0.0009936 M. AND THE IONIC STRENGTH VARIED BY ADDITION OF POTASSIUM CHLORIDE

| KCL MOL/L. | $\sqrt{\mu}$ | $P_{CO_2}$ | $pP_{CO_2}$ | pH | $pHCO_3$ | $pf_{HCO_3}$ | pK |
|---|---|---|---|---|---|---|---|
| 0.01 | 0.1048 | 38.04 | 1.301 | 6.012 | 3.003 | 0.048 | 6.419 |
| " | " | 152.7 | 0.697 | 5.403 | " | " | 6.414 |
| " | " | 743.4 | 0.009 | 4.726 | " | " | 6.425 |
| 0.10 | 0.3178 | 38.0 | 1.301 | 5.940 | " | 0.125 | 6.424 |
| " | " | 152.6 | 0.697 | 5.330 | " | " | 6.418 |
| " | " | 743.4 | 0.009 | 4.656 | " | " | 6.432 |
| 0.25 | 0.501 | 38.05 | 1.300 | 5.888 | " | 0.178 | 6.426 |
| " | " | 152.9 | 0.697 | 5.276 | " | " | 6.417 |
| " | " | 744.6 | 0.009 | 4.612 | " | " | 6.441 |
| | | | | | | AVERAGE | 6.4240 |
| | | | | | | GENERAL AVERAGE | 6.4252 |

The average value for the change in pH with change in $pP_{CO_2}$ was found to be 0.995 which conforms well with the theoretical value of unity (fig. 6).

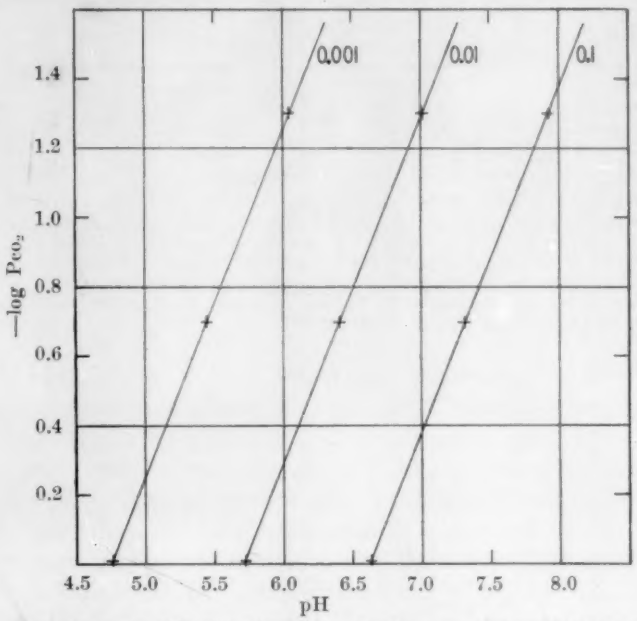



FIG. 6. The change in pH with change in $-\log P_{CO_2}$ at various bicarbonate ion concentrations (0.1, 0.01, and 0.001 M × 0.9936).

CONCENTRATION OF BICARBONATE ION IN SUNFLOWER-LEAF SAP.—The bicarbonate ion concentration of sunflower-leaf sap was calculated from the equation for the primary ionization constant of carbonic acid, pK 6.425. All other terms in the equation were measured in the manner already described with the exception of the activity coefficient of the bicarbonate ion. This was estimated by an independent experiment.

For the present it is assumed that the activity coefficient of the bicarbonate ion in the sap depends chiefly upon the concentrations of the inorganic ions. To determine the concentrations of these ions, a known volume of sap was ashed and the principal inorganic ions estimated by the usual methods of analysis. The concentrations so determined, expressed in mols per liter, are shown in table VIII. The organic ions were of course destroyed by the ashing process. The solubility of calcium phosphate would be exceeded with such concentrations of calcium and phosphate ions present in the sap. This indicates that at least a part of these ions is bound in little-ionized or non-ionized forms.

By mixing the ions in the proportions obtained by analysis (supplying organic ions as acetate and formate) a "synthetic sap" was prepared which

TABLE VIII

CONCENTRATION OF IONS IN SUNFLOWER-LEAF SAP

| CATION | CONCENTRATION MOLS/LITER | ANION | CONCENTRATION MOLS/LITER |
|---|---|---|---|
| Calcium | 0.01306 | Phosphate | 0.0166 |
| Magnesium | 0.01002 | Sulphate | 0.0274 |
| Potassium | 0.1596 | Chloride | 0.0363 |
| | | Organic* | 0.0815 |

* The organic anion concentration was calculated as the difference in the number of equivalents excess of cations over anions and includes bicarbonate ion.

Note.—Allowing for the solubility of calcium phosphate as 0.561 gm. per liter of water saturated with carbon dioxide (20) the ionic strength of the sap was calculated as 0.2434. Applying the formula of GUGGENHEIM and SCHINDLER (10) and calculating the specific effect of the cations to be the same as an equal concentration of potassium ions, a value of $pf_{HCO_3} = 0.173$ was obtained.

was free from any compound that might bind carbon dioxide in any way except by neutralization. When the components were mixed, a precipitate appeared which was dissolved by bubbling carbon dioxide through the solution. On standing over night, calcium phosphate crystallized out of the solution. After standing at room temperature for several days the crystals were removed by filtration. The supernatant liquid was saturated with carbon dioxide at known partial pressures in the gas reaction cell (fig. 5) and the activity of the bicarbonate ion determined. The value of the activity coefficient found was $f_{HCO_3} = 0.665$ ($pf_{HCO_3} = 0.177$). A closely-agreeing value $f_{HCO_3} = 0.662$ ($pf_{HCO_3} = 0.179$) was obtained also by the use of a similar solution containing glucose (approx. 0.176 M).

The bicarbonate concentrations used in calculating the activity coefficients of bicarbonate ions were determined by means of the VAN SLYKE and NEILL blood-gas analysis apparatus (31). Since the solutions analyzed were saturated with carbon dioxide it was necessary to subtract the amount of dissolved carbon dioxide in order to obtain the bicarbonate concentration. This solubility was estimated by an independent measurement of the solubility of carbon dioxide in the solution. For this determination the solution was slightly acidified (pH 3.47) with concentrated hydrochloric acid.

An independent estimate of the activity coefficient of the bicarbonate ion was made from the ionic strength of the solution. The value calculated, $f_{HCO_3} = 0.671$ ($pf_{HCO_3} = 0.173$), corroborated the value obtained by direct experiment.

The following equation was obtained by substituting these experimentally determined values:

$$p_{HCO_3} = 6.425 + 1.343 - 0.177 + pP_{CO_2} - pH$$

This reduces to the simplified expression

$$p_{HCO_3} = 7.591 + pP_{CO_2} - pH$$

In table IX and figure 7 the concentrations of bicarbonate ion calculated from this equation are compared with the total amount of bicarbonate bound

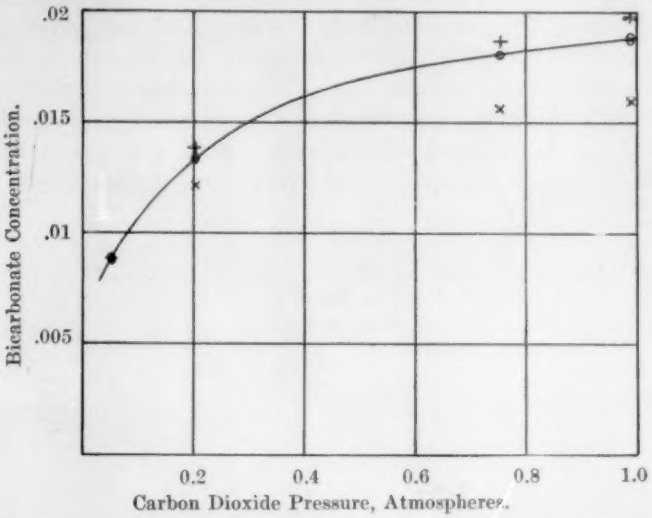



FIG. 7. The bicarbonate ion concentration determined from electromotive force measurements (+) compared with the total combined carbon dioxide obtained by gas-analytical methods (O) and that calculated from the buffer action of the phosphates (X) in sunflower-leaf sap.

## TABLE IX

BICARBONATE-ION CONCENTRATION OF SUNFLOWER-LEAF SAP

| $P_{CO_2}$ | $pP_{CO_2}$ | pH | $pHCO_3$ | $HCO_3$ (E.M.F.) | $HCO_3$ (ANAL.) |
|---|---|---|---|---|---|
| *atm.* | *atm.* | | *mol/l.* | *mol/l.* | *mol/l.* |
| 0.05065 | 1.295 | 6.831 | 2.055 | 0.00881 | 0.00875 |
| 0.05063 | 1.296 | 6.831 | 2.056 | 0.00879 | 0.00886 |
| 0.2030 | 0.693 | 6.424 | 1.860 | 0.01380 | 0.01326 |
| 0.2030 | 0.693 | 6.424 | 1.860 | 0.01380 | 0.01339 |
| 0.7536 | 0.123 | 5.984 | 1.730 | 0.01863 | 0.01801 |
| 0.7533 | 0.123 | 5.984 | 1.730 | 0.01863 | 0.01802 |
| 0.9881 | 0.005 | 5.891 | 1.705 | 0.01973 | 0.01867 |
| 0.9882 | 0.005 | 5.892 | 1.704 | 0.01977 | 0.01884 |

chemically by the sap as determined by analytical means (19). This comparison shows that the two sets of values are nearly equal. The conclusion may be drawn, therefore, that all of the combined carbon dioxide in the leaf sap was present as bicarbonate ion and that none of it had added directly to the sap constituents to form carbamino-like compounds.

THE CARBON DIOXIDE ABSORBENTS IN SUNFLOWER-LEAF SAP.—From the foregoing experiments it is clear that, in sunflower-leaf sap, buffer sub-

stances are present which are capable of reacting with carbon dioxide; otherwise the bicarbonate ion concentration would not increase with increase in pressure of carbon dioxide.

By correlating the phosphate content with the neutralization capacity of the sap from the hypocotyl of sunflower plants, MARTIN (**15**) concluded that the buffer action was due almost entirely to phosphates. From the experiments on sunflower leaves reported here it is apparent that the increase in bicarbonate ion concentration with increase in carbon dioxide pressure is too large to be accounted for solely by the phosphates (table X, fig. 7). Also

TABLE X

CHANGE IN SECONDARY-PHOSPHATE CONCENTRATION IN SUNFLOWER-LEAF SAP WITH CHANGE IN PRESSURE OF CARBON DIOXIDE*

| pH‡ | $-\log \frac{HPO_4^=}{H_2PO_4^-}$ | $\frac{HPO_4^=}{H_2PO_4^-}$ | $HPO_4^=$ | $P_{CO_2}$ | $HCO_3$† (CALC.) | $HCO_3$† (OBS.) |
|---|---|---|---|---|---|---|
| 6.831 | −0.115 | 1.303 | 0.009390 | 0.0507 | (0.00875) | 0.00875 |
| 6.424 | 0.292 | 0.5105 | 0.005611 | 0.2030 | 0.01213 | 0.01326 |
| 5.984 | 0.732 | 0.1854 | 0.002596 | 0.7536 | 0.01554 | 0.01801 |
| 5.891 | 0.825 | 0.1496 | 0.002160 | 0.9881 | 0.01598 | 0.01867 |

* In these calculations the value of $\mu = 0.2434$, was taken as the ionic strength of the sap.

The calculations were then made from the formula

$$-\log \frac{HPO_4^=}{H_2PO_4^-} = 7.211 - 0.495 - pH$$

† $HCO_3$ calculated is the sum of the bicabonate ion concentration determined analytically at 0.05 atmospheres carbon dioxide plus the increase calculated from the change in primary phosphate ion.

$HCO_3$ observed was determined by means of the blood-gas analysis apparatus.

‡ The pH measurements in this paper are referred to the pH values for standard buffers given by MACINNES, BELCHER, and SHEDLOVSKY (**14**): for the standard 0.1 N acetate buffer pH 4.650, and for the potassium acid phthalate, 0.05 M, pH 4.000.

the neutralization of the sap by acid (fig. 8) demonstrates that the sap contains other neutralizing substances. While phosphates may be the chief buffers, other buffer substances are present which have not yet been identified.

From separate experiments the second ionization constant of phosphoric acid, on which the calculations for table X and figure 7 are based, was found to be defined by the following equation:

$$pK = pH - \log \frac{HPO_4^=}{H_2PO_4^-} + \frac{1.5\sqrt{\mu}}{1+\sqrt{\mu}} \qquad (9).$$

The value estimated for pK is 7.211 at 15.0° C. (table XI).[5]

By determining the pH and applying this equation, the change in the

[5] A value pK = 7.228 (15.0°) was estimated from the values given by NIMS (**16**) extrapolated to 15.0° by the formula of HARNED and EMBREE (**11**).

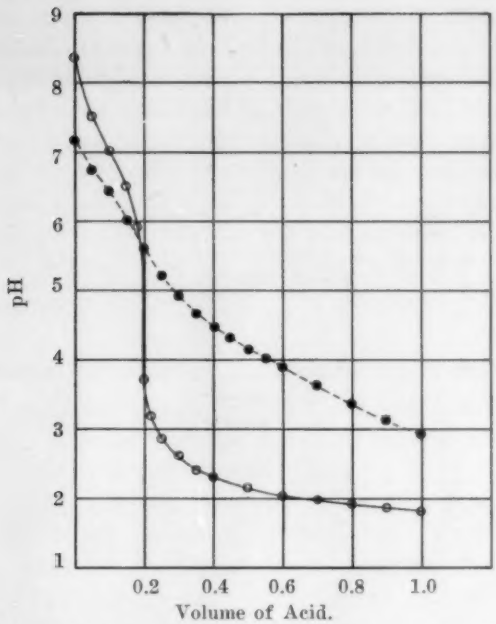



FIG. 8. Electrometric titration curves of sunflower-leaf sap ( • ) and of 0.02 M dipotassium phosphate ( O ). Ordinate, pH; abscissa, volume of N/1 × 0.9685 hydrochloric acid. (10.00 ml. of each solution were used.)

ratio of secondary to primary phosphate was calculated. From this ratio and the total phosphate content of the sap the increase in bicarbonate ion concentration was determined. This was found to be considerably less than the increase in bicarbonate ion concentration determined analytically (table X).

The method of estimating bicarbonate ion concentration, from the trans-

TABLE XI

DETERMINATION OF THE IONIZATION CONSTANT OF THE DIHYDROGEN PHOSPHATE ION. THE EFFECT OF POTASSIUM CHLORIDE. RATIO OF SECONDARY TO PRIMARY PHOSPHATE = 1.0; TOTAL CONCENTRATION OF PHOSPHATE ION = 0.01 M; TEMPERATURE, 15.0° C.

| CONC. KCl MOL/L. | $\mu_{KCl}$ | $\mu_{PO_4}$ | $\mu_{TOTAL}$ | $\frac{1.5\sqrt{\mu}}{1.0+\sqrt{\mu}}$ | pH | pK |
|---|---|---|---|---|---|---|
| 0.0 | 0.0 | 0.02 | 0.020 | 0.185 | $7.02_5$ | 7.210 |
| 0.10 | 0.10 | 0.02 | 0.120 | 0.386 | 6.82 | 7.206 |
| 0.20 | 0.20 | 0.02 | 0.220 | 0.478 | $6.73_3$ | 7.211 |
| 0.50 | 0.50 | 0.02 | 0.520 | 0.628 | $6.58_5$ | 7.213 |
| 0.85 | 0.85 | 0.02 | 0.870 | 0.723 | 6.49 | 7.213 |
| | | | | | AVERAGE | 7.211 |

formation of secondary into primary phosphate ion, was verified by a separate experiment. A solution of dipotassium hydrogenphosphate (0.0184 M) was saturated with carbon dioxide. From the pH (5.908) of the resultant solution the change in concentration of secondary phosphate was calculated. The amount of secondary phosphate transformed was found to be equal to the increase in bicarbonate ion determined either by the analytical method of VAN SLYKE and NEILL (31) or by the ionization constant equation for carbonic acid. A comparison of the values obtained by these independent methods is here tabulated:

| Method used | Equation $K_{H_2PO_4^-}$ | Equation $K_{H_2CO_3}$ | Gas-analytical |
|---|---|---|---|
| Bicarbonate concentration obtained ..... | 0.01693 | 0.01648 | 0.01635 |

These results justified the methods of calculation used for the estimation of bicarbonate ion concentration in sunflower-leaf sap.

In summarizing, it may be said that phosphates probably play an important rôle in the buffer action of sunflower-leaf sap toward carbonic acid; other substances are present, however, in the sap which react with carbonic acid. This was demonstrated by obtaining the neutralization curve of sunflower-leaf sap and also by calculating the amount of carbonic acid that could be neutralized by the quantity of secondary phosphate transformed.

### ABSORPTION OF CARBON DIOXIDE BY THE WATER-INSOLUBLE LEAF RESIDUE

The killed leaf, even after extraction with water, absorbed carbon dioxide. The ash of this solid leaf residue contained calcium and magnesium salts, the amounts of which accounted completely for the alkalinity of the ash (table XII). Inasmuch as the extracted leaf residue liberated carbon

TABLE XII

BASICITY OF THE ASH FROM THE INSOLUBLE SUNFLOWER-LEAF RESIDUE

| | |
|---|---|
| Gram atoms of calcium | $6.69 \times 10^{-4}$ |
| Gram atoms of magnesium | $1.80 \times 10^{-4}$ |
| Sum | $8.49 \times 10^{-4}$ |
| Equivalents of base | $16.98 \times 10^{-4}$ |
| Equivalents of acid used for neutralization | $16.57 \times 10^{-4}$ |

dioxide when treated with cold dilute hydrochloric acid, the active absorptive agents in the residue were probably calcium and magnesium carbonates. If such were the case, an extraction of the leaf residue with water saturated with carbon dioxide would remove these salts. This would decrease the calcium and magnesium content of the residue and at the same time remove its ability both to absorb carbon dioxide and also to liberate carbon dioxide

on treatment with acid. Furthermore, alkaline-earth carbonates would be found in the extract. This prediction was verified as shown in table XIII.

## TABLE XIII

RELATION OF CARBON DIOXIDE ABSORPTION TO ALKALINE EARTH CARBONATE CONTENT. QUANTITIES BASED ON 10.00 GRAMS OF FRESH LEAVES. ALL QUANTITIES ARE EXPRESSED IN MOLS $\times 10^4$

| SAMPLE NUMBER | | FRESH LEAF | FROZEN LEAF | RESIDUE FROM $H_2O$ EXTRACTION | WATER EXTRACT | RESIDUE FROM $H_2O$–$CO_2$ EXTRACTION | $H_2O$–$CO_2$ EXTRACT |
|---|---|---|---|---|---|---|---|
| 1 | Reversible $CO_2$ | 1.34 | 3.99 | 2.19 | 2.17 | 0.20 | 2.67 |
| 2 | Irreversible $CO_2$ | 7.09 | 3.52 | 1.92 | −0.17 | 0.00 | 2.38 |
| 3 | Combined $CO_2$ | 8.43 | 7.51 | 4.12 | 2.00 | 0.20 | 5.05 |
| 4 | Calcium | 5.59 | 6.18 | 6.48 | 0.78 | 3.29 | 2.92 |
| 5 | Magnesium | 2.38 | 2.46 | 1.69 | 0.87 | 0.49 | 1.03 |
| 6 | Calcium + magnesium | 7.97 | 8.64 | 8.17 | 1.65 | 3.78 | 3.95 |

These data make it apparent that a close correlation exists between the content of alkaline earths and the amount of carbon dioxide that can be combined by the leaf residue.

In order to confirm the observation that water saturated with carbon dioxide removed calcium and magnesium carbonates from water-insoluble leaf residue, the dissolved material was isolated in solid form.

Frozen sunflower-leaf material (210 gm.) was prepared as has already been described. Water-soluble material was removed by two extractions with water (2500 ml. each). The insoluble residue was then extracted twice with water saturated with carbon dioxide (2500 ml. each). The carbonic acid extracts were concentrated to 1500 ml. by boiling and the solid which precipitated was filtered off. This solid was extracted with two portions of water saturated with carbon dioxide (500 ml. each). By boiling this extract a solid was again precipitated which was collected and dried. It weighed 0.2459 gm.

Analysis showed the precipitate to have the following composition:

| Constituent | Per cent. | Constituent | Per cent. |
|---|---|---|---|
| CaO | 51.45 | $CO_2$ | 33.39 |
| MgO | 1.29 | Volatile not $CO_2$ | 3.47 |
| MnO | 0.65 | | |
| $P_2O_5$ | 10.86 | Total | 101.11 |

This solid material absorbed carbon dioxide and also liberated carbon dioxide when treated with acid. 0.0257 gm. of this material liberated 4.34 ml. of carbon dioxide when treated with acid. The same quantity of solid absorbed 1.83 and 2.40 ml. of carbon dioxide at 0.678 and 0.891 atmospheres pressures of carbon dioxide, respectively (volumes reduced to 0°, 760 mm.).

It has been demonstrated by analytical and preparative procedures that the absorptive agents in the water-insoluble residue from sunflower leaves are calcium and magnesium carbonates and perhaps phosphates. The presence of manganese in the carbonate precipitates is noteworthy in view of the recent experiments which show that manganese may play a rôle in the absorption of carbon dioxide by water plants (**2**) and, under certain conditions, may stimulate photosynthesis (**17**).

### Alkalinity of saps from leaves treated with carbon dioxide

Leaves from some plants when placed in an atmosphere containing high concentrations of carbon dioxide yield an expressed sap which is more alkaline than the sap from leaves maintained under normal conditions (**30**). Fife and Frampton (**8**) investigated this phenomenon under a variety of conditions and concluded that under the influence of high concentrations of carbon dioxide the plants catalyzed the conversion of acid amides into ammonium salts and on removal of the carbon dioxide the plants catalyzed the reverse reaction.

$$2\ RCONH_2 + 3H_2O \underset{(-CO_2)}{\overset{(+CO_2)}{\rightleftharpoons}} RCOONH_4 + RCOOH + NH_4^+ + OH^-$$

This hypothetical mechanism was supported by the facts that the quantity of ammonia nitrogen increased and amide nitrogen decreased when the leaves were placed in an atmosphere of high carbon dioxide content, whereas the reverse occurred when the leaves were transferred to ordinary air.

In view of the results reported in the preceding section it seemed probable that the formation of alkaline-earth bicarbonates might also be involved in this phenomenon.

Water charged with carbon dioxide removes calcium and magnesium carbonates from the water-insoluble sunflower-leaf residue. When the leaf is stored in high concentratitons of carbon dioxide gas carbonic acid concentration of the sap will increase and dissolve additional amounts of the alkaline-earth carbonates. The soluble calcium and magnesium bicarbonates so formed will be expressed with the sap and increase the pH of the sap by repressing the acidity of the carbonic acid according to the equation:

$$[H^+] = \frac{K[H_2CO_3]}{[HCO_3^-]}.$$

To test this hypothesis three lots of sunflower leaves were frozen

(−70° C.) in three different atmospheres: one lot in air; one in nitrogen; and one in carbon dioxide. The sap from each lot was pressed out and the pH values of the sap measured under the following conditions: just as the sap came from the press; after being swept with nitrogen gas; after being saturated with carbon dioxide gas. The data from these experiments are recorded in table XIV.

TABLE XIV

ALKALIZATION OF SUNFLOWER-LEAF SAP

| | Leaves treated with | | |
|---|---|---|---|
| | Nitrogen | Air | Carbon dioxide |
| Original pH of sap | 6.82 | 6.82 | 7.02 |
| Saturated with carbon dioxide | 6.06 | 6.03 | 6.38 |
| Sap flushed with nitrogen | 7.07 | 7.13 | 8.22 |
| Molarity in sap: | | | |
| Calcium | 0.0204 | 0.0206 | 0.0250 |
| Magnesium | 0.0211 | 0.0216 | 0.0230 |

The sap expressed from the carbon dioxide-treated leaves is less acid than the saps from leaves treated with either air or nitrogen. This result confirms the observations of FIFE and FRAMPTON. The sap expressed from leaves treated with carbon dioxide becomes less acid when saturated with carbon dioxide gas than do the saps from leaves treated with air and with nitrogen. When flushed with nitrogen the sap from carbon dioxide-treated leaves becomes more alkaline than the saps from the nitrogen or air-treated leaves. Treatment of leaves with carbon dioxide increases the calcium and magnesium content of the sap.

These observations can be correlated by assuming that alkaline earth carbonates are removed from the structural material of the leaf and dissolved in the leaf sap as bicarbonates by treatment with carbon dioxide. Undoubtedly this reaction also is involved in the alkalization of sunflower-leaf sap by treatment of the leaf with carbon dioxide.

What relation the absorption of carbon dioxide by the alkaline-earth carbonates bears to photosynthesis in land plants is not known. If, however, land plants can utilize carbon dioxide bound to the alkaline earths as well as water plants do, this mechanism of carbon dioxide absorption may be of considerable significance to them (**1**).

### ABSORPTION OF CARBON DIOXIDE BY CHLOROPHYLL

One of the intriguing questions concerning photosynthesis is how the energy absorbed by chlorophyll is used to reduce carbon dioxide. Chemical

combination between chlorophyll and carbon dioxide has been one answer. It is of interest, therefore, to determine whether any evidence exists from previous work for such a combination. Two methods have been used to obtain such evidence. One method has been to determine whether chlorophyllous tissues absorbed more carbon dioxide than did non-chlorophyllous tissues; the other method has been to find out whether isolated chlorophyll and its derivatives exhibited any tendency to react with carbon dioxide. The results obtained previously have not been conclusive and in some cases have been actually contradictory. Because of the importance of the conception of a pigment-carbon-dioxide complex to the formulation of a proper scheme for the mechanism of photosynthesis, further evidence regarding the existence of such a complex has been sought.

In regard to the absorption of carbon dioxide by green and yellow varieties of leaves WILLSTÄTTER and STOLL (33b) obtained no difference between such varieties of elder and elm. On the other hand SPOEHR and MCGEE

TABLE XV

CARBON DIOXIDE ABSORPTION* BY CHLOROPHYLLOUS AND NON-CHLOROPHYLLOUS LEAVES

| No. | TYPE OF VARIANT | SPECIES | CHLOROPHYLLOUS | NON-CHLOROPHYLLOUS | $CO_2$-EQUIV. CHLOROPHYLL (GREEN VARIETIES) | pH OF CHLOROPHYLLOUS | pH OF NON-CHLOROPHYLLOUS |
|---|---|---|---|---|---|---|---|
| | | | *ml.* | *ml.* | *ml.* | | |
| 1 | Etiolated | *Nicotiana tabacum* | 1.02 | 0.34 | | | |
| 2 | " | *Hordeum vulgare* | 1.09 | 0.65 | 0.38 | 5.78 | 5.77 |
| 3 | " | " | 0.70 | 0.75 | 0.39 | 5.78 | 5.78 |
| 4 | " | *Phaseolus multiflorus* | 1.30 | 2.81 | | | |
| | " | *Zea mays* | 1.19 | 0.85 | 0.64 | 5.72 | 5.62 |
| 6 | " | *Pisum sativum* | 1.44 | 0.85 | 0.50 | 5.92 | 5.62 |
| 7 | Aureous variety | *Evonymus japonicus* | 0.92 | 1.52 | 0.41 | 5.05 | 5.42 |
| 8 | Albino | *Zea mays* | 1.41 | 1.55 | | 5.78 | 5.75 |
| 9 | " | " " | 1.02 | 1.37 | 0.36 | 5.52 | 5.68 |
| 10 | Yellow with age | *Nicotiana tabacum* | 1.02 | 0.03 | 0.39 | 5.68 | 5.41 |

* The absorption values are given as cubic milliliters of $CO_2$, (0°, 760 mm.) absorbed at one atmosphere of $CO_2$-pressure by 10.00 grams of fresh leaf material, in excess of that ascribable to water. The $CO_2$-equivalent of chlorophyll was based on the ratio of one mol of carbon dioxide to one mol of pigment. Measurements were made by the use of apparatus shown in figure 1.

(25) found indication that "green leaves and stems absorb considerably more carbon dioxide than the corresponding etiolated portions."

The effect of chlorophyll on the absorption of carbon dioxide by leaves has been re-examined. It has been found that sometimes chlorophyllous and sometimes non-chlorophyllous leaves absorb the more carbon dioxide. No consistency has been obtained (table XV).

In order to gain a more sound basis of comparison between chlorophyllous and non-chlorophyllous leaf material, the absorption of carbon dioxide by the white and by the green portions of the same variegated ivy leaves was measured. The colorless portions absorbed 1.3 ml. and the green parts 0.94 ml. of carbon dioxide per unit weight at 0.25 atmospheres of pressure, in excess of that ascribable to the water present. This experiment confirmed the results obtained by the use of other non-chlorophyllous materials.

Further evidence regarding the effect of chlorophyll was obtained in the following way. 10.00 gm. of fresh leaves were frozen, thawed, and extracted with water charged with carbon dioxide. This removed all water-soluble carbon dioxide absorbents, but left the chlorophyll in the leaf residue intact. This chlorophyll-containing residue absorbed no more carbon dioxide (0.21 ml.) than did a similar residue from which the chlorophyll had been extracted with alcohol (0.27 ml.). The amount absorbed in both cases was insignificant. No carbon dioxide was liberated with cold dilute acid from either sample.

From these experiments it may be concluded that chlorophyll is not the factor controlling the absorption of carbon dioxide by leaves and no evidence has been obtained that the chlorophyll in leaves unites with carbon dioxide.

Previous experiments with isolated chlorophyll had indicated that chlorophyll united with carbon dioxide. The experiments of WILLSTÄTTER and STOLL demonstrated that colloidal chlorophyll in water suspension absorbs carbon dioxide (33d). Most of the absorbed carbon dioxide could be accounted for by the reaction with, and removal of, the magnesium in the pigment. There was, however, an additional absorption which was attributed to the pigment itself.

A reinvestigation of this reaction (carried out at 25.1° instead of 0°, the temperature used by WILLSTÄTTER and STOLL) confirmed the observation that carbon dioxide is absorbed and magnesium removed from the pigment. The amount of carbon dioxide absorbed in excess of that calculated for the water was less than the amount necessary to form magnesium bicarbonate with the magnesium removed from the pigment.

From the reaction rate curve (fig. 9) it is apparent that the reaction had not reached completion. The rate of absorption had become so slow, because of the flocculation of the pigment, that it seemed unprofitable to con-

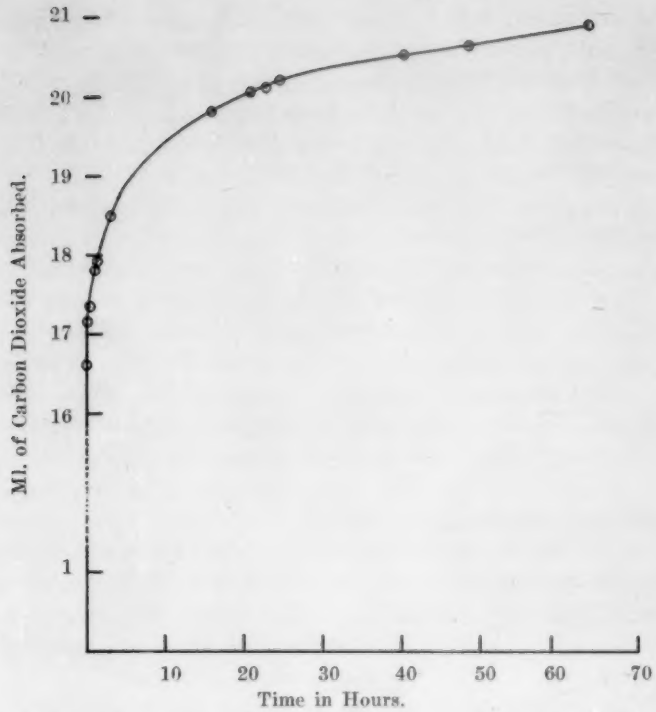



FIG. 9. Curve representing the rate of absorption of carbon dioxide by an aqueous suspension of colloidal chlorophyll.

tinue the experiment longer. A summary of the results is given in table XVI.

## TABLE XVI

ON THE ABSORPTION OF CARBON DIOXIDE BY COLLOIDAL CHLOROPHYLL AND THE SPLITTING OUT OF THE MAGNESIUM BY THE CARBON DIOXIDE

| | |
|---|---|
| Volume of solution of colloidal chlorophyll (ml.) | 20.45 |
| Weight of colloidal chlorophyll (gm.) | 0.32 |
| Volume of chlorophyll (ml.) | 0.29 |
| Volume of water (ml.) | 20.13 |
| Pressure of $CO_2$ (mm.) | 737.2 |
| Temperature (0° C.) | 25.1 |
| Vol. $CO_2$ (0°, 760 mm.) required by water (ml.) | 14.82 |
| Vol. $CO_2$ dissolved by suspension (ml.) | 20.85 |
| Vol. of $CO_2$ reduced to standard conditions (ml.) | 18.52 |
| Excess vol. dissolved by suspension (ml.) | 3.70 |
| Weight of $CO_2$ dissolved (mg.) | 7.31 |
| Mols of $CO_2$ dissolved $\times 10^4$ | 1.661 |
| Atoms of magnesium removed $\times 10^4$ | 1.114 |
| Ratio $CO_2$/Mg | 1.491 |

The chlorophyll used in this experiment was obtained by the method of

WILLSTÄTTER and STOLL (32a). The colloidal suspension was prepared by HUBERT's method (12), 0.5 gm. of chlorophyll being dispersed in 32 ml. of colloidal suspension. HUBERT's method was modified only in that the acetone solution of chlorophyll was forced into the rapidly stirred water by gravity rather than by compressed air.

The absorption of carbon dioxide by the colloidal suspension of chlorophyll was measured in the apparatus diagrammed in fig. 10. The apparatus

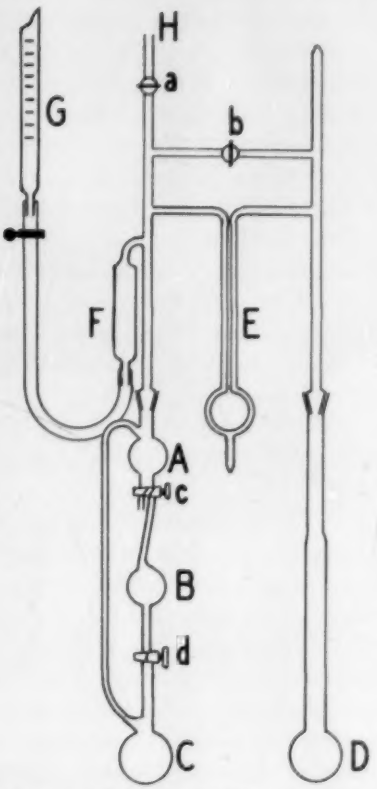



FIG. 10. Apparatus for measuring the solubility of carbon dioxide in a colloidal suspension of chlorophyll in water.

was patterned after the one previously used in this laboratory for microhydrogenation (22).

The suspension was introduced into bulb A. With stopcocks *a*, *b*, and *d* open and *c* closed, the air was removed from the apparatus and the dissolved gases pumped out of the colloidal suspension. Stopcock *d* was then closed and *c* opened so that the suspension ran into the calibrated pipet B. Stopcock *a* was opened and air allowed to enter. The apparatus was again

evacuated and any bubbles that formed in B removed. This alternate evacuation and filling with air was continued until pipet B was completely full of liquid. Stopcock *c* was then turned so as to drain the excess solution out of bulb A. When all had drained out, *c* was closed. The apparatus was then alternately evacuated and filled with carbon dioxide until it contained pure carbon dioxide. The whole apparatus was lowered into the constant temperature bath (25.1° C.) and shaken until the water contained in D and the rest of the apparatus was saturated with carbon dioxide. Stopcock *b* was closed and as soon as the manometer liquid in the two arms of the manometer E had remained level for a half-hour the solution was allowed to flow into the reaction bulb C by opening stopcocks *c* and *d*. Absorption was evidenced by the change in level of the manometer liquid. To equalize the levels of the liquid in the two arms of the manometer, mercury was let into the reservoir F from buret G. When equilibrium was established, as shown by the constancy of the manometer, the volume of the gas absorbed was read from the buret.

The auxiliary apparatus connected to the absorption apparatus at H is not pictured. It consisted of a carbon dioxide generator, washflasks, manometer, and pumps so arranged with stopcocks that the evacuation and filling of the absorption apparatus with the desired gases could be effected readily.

This absorption apparatus gave the accepted value for the Bunsen absorption coefficient of carbon dioxide in water, 0.753 at 25.1° C. Bohr found the value, 0.757 (**3**).

The experimental data for the absorption of carbon dioxide by the colloidal suspension of chlorophyll are given in table XVI and figure 9.

During the period of absorption, 64 hours, the colloidal chlorophyll flocculated and precipitated out. As soon as the experiment was completed the reaction vessel was removed from the apparatus, the solution filtered from the separated chlorophyll, and the magnesium in the filtrate estimated as the 8-hydroxyquinolate; the precipitated magnesium complex was determined gravimetrically. The number of atoms of magnesium recovered was $1.114 \times 10^{-4}$. The number of mols of chlorophyll used was $3.54 \times 10^{-4}$. Therefore, not all of the magnesium had been removed from the chlorophyll.

Recalculation of the results of Willstätter and Stoll (**33g**) showed that the solubility of carbon dioxide in suspensions of colloidal chlorophyll exceeded that necessary to saturate the water and to form magnesium bicarbonate by about 0.1 mol of carbon dioxide per mol of pigment. The solubility of carbon dioxide in the solid pigments approaches this value. This suggests that the results of Willstätter and Stoll may be explained by the solubility of the carbon dioxide in the solid pigment phase in the colloidal suspension, in which case no evidence remains for the chemical union between chlorophyll and carbon dioxide.

RABINOWITCH (**18**) has recently reported that solid ethylchlorophyllide absorbs carbon dioxide. The extrapolated limit of absorption is two mols of carbon dioxide to one of pigment. This value is suggestive of compound formation between chlorophyll and carbon dioxide.

To ascertain whether evidence also exists for compound formation between chlorophyll and carbon dioxide, the solubility of carbon dioxide in solid chlorophyll (a + b) and in pheophytin (a + b) was measured. Within the limits of accuracy of the apparatus the absorption appeared to be directly proportional to the pressure of the carbon dioxide (fig. 11). This

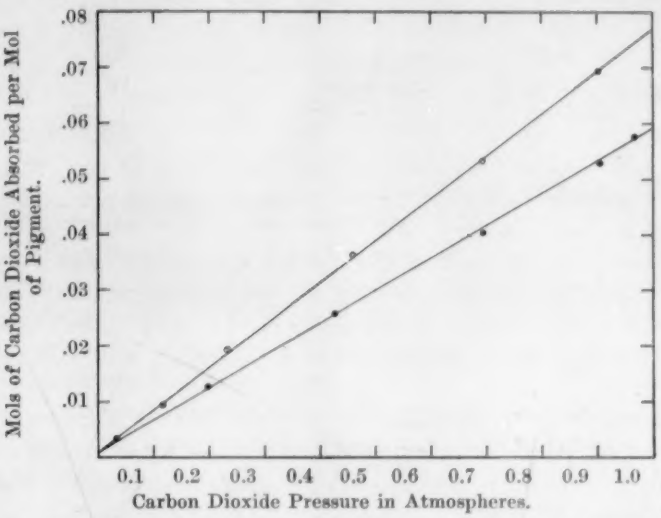



FIG. 11. The absorption of carbon dioxide by chlorophyll and pheophytin in the solid state. Temperature 0.1° C.

Ordinate: mols of carbon dioxide absorbed per mol of pigment. Abscissa: pressure of carbon dioxide in atmospheres. Chlorophyll ●; pheophytin O.

pointed to physical solution rather than to chemical combination of the carbon dioxide. When pheophytin was not thoroughly dry a slight deviation from linearity was obtained which may have been the result of chemical action.

The absorption of carbon dioxide by solid chlorophyll was carried out at 0.1° C. in the apparatus shown in figure 1. The chlorophyll had been prepared from mallow leaves by the method of WILLSTÄTTER and STOLL (**32**d). It contained 2.68 per cent. magnesium.

The method of measurement was the same as that which has already been described except that the free volume of the reaction vessel (49.54 ml.) was calculated from the known volume of the vessel and the volume of the chlorophyll. [The weight used was 3.2022 gm. and the density taken was 1.11, the value given by KETELAAR and HANSON (**13**)].

Determination of the free volume of the reaction vessel by admission of nitrogen and hydrogen gave values which were larger than that calculated from the geometry of the system (nitrogen, 49.66; hydrogen, 49.72 ml.). On the assumption that the larger values were caused by the solubility of these gases in the pigment, solubility coefficients of nitrogen and hydrogen in chlorophyll were calculated. A comparison of the solubilities of nitrogen, hydrogen, and carbon dioxide in chlorophyll at 0.1° C. and 760 mm. pressure are tabulated:

| Gas | Solubility ml. |
|---|---|
| Nitrogen | 0.0387 |
| Hydrogen | 0.0547 |
| Carbon dioxide | 1.472 |

The solubilities are expressed as cubic milliliters of gas absorbed (reduced to standard conditions) by one gram of pigment per atmosphere of pressure of carbon dioxide.

The absorption of carbon dioxide by chlorophyll was very rapid. Within five minutes after admission of the gas, equilibrium was established. The absorption was completely reversible: the volume absorbed was 4.57 ml. (manometrically); the volume removed by pumping was 4.59 ml. (volumetrically).

The absorption of carbon dioxide by pheophytin was measured in the same way as described for chlorophyll. The solubility of carbon dioxide in pheophytin was found to be 1.963 ml. per gm. of pigment per atmosphere of carbon dioxide pressure.

Pheophytin was prepared from the chlorophyll used in the previous experiments. The chlorophyll was dissolved in 200 ml. of 95 per cent. ethanol. To this solution 12 ml. of water and 1 ml. of concentrated hydrochloric acid were added and the mixture shaken for thirty minutes. The pheophytin which precipitated was filtered off and washed with distilled water until the washing no longer reacted acid to litmus. The pigment was dried over calcium chloride, then in an Abderhalden pistol at the boiling point of methyl alcohol and finally stored over calcium chloride in a vacuum desiccator for two days. This material contained 0.22 per cent. ash.

According to WILLSTÄTTER and STOLL (32b), pheophytin is not extracted from ether solution with hydrochloric acid less concentrated than 25 per cent. and is almost completely extracted by 32 per cent. Only a trace of this pigment was removed from ether solution by 22 per cent. hydrochloric acid and a large proportion was removed by 37 per cent. acid. From these tests it was concluded that the pigment still contained the phytyl group.

During its transfer to the reaction vessel, the pigment appeared to take up moisture. To remove this moisture the reaction vessel containing the pigment was warmed (40° to 45° C.) and alternately pumped out with the Sprengel pump and flushed with dry nitrogen. The sweeping process was continued until the volume of the nitrogen admitted to the reaction vessel (measured manometrically) was the same as the volume recovered by pumping.

The absorption was directly proportional to the pressure of the carbon dioxide (fig. 11).

### Liberation of carbon dioxide with boiling hydrochloric acid

One question concerning the absorption of carbon dioxide by unilluminated leaves is whether other compounds besides carbonates and bicarbonates exist in leaves which can make carbon dioxide easily available to the leaf. An attempt was made to answer this question by determining the amounts of carbon dioxide liberated from leaves by boiling the leaves with hydrochloric acid of different concentrations.

Tobacco leaves, which had been stored in the dark for four days, were used in these experiments. Samples (25 gm.) of the parenchymatous tissues were cut from the mid-rids and put into a flask containing 125 ml. of the acid solution to be tested. The leaf material was boiled for one hour and the liberated carbon dioxide measured.

Experiments were performed with solutions containing 0, 1, 5, and 12 per cent. hydrochloric acid, respectively. The amounts of carbon dioxide liberated were:

| Hydrochloric acid used, per cent. | 0 | 1 | 5 | 12 |
|---|---|---|---|---|
| Carbon dioxide liberated, mg. ..... | 6.62 | 9.95 | 16.18 | 51.70 |

The periods of boiling were probably too short to produce the maximum quantities of carbon dioxide, but it is evident that carbon dioxide may be liberated from leaf material with different degrees of facility. There was some evidence that storage of the leaves depleted the amount of carbon dioxide that could be liberated with 12 per cent. hydrochloric acid.

## Summary

Measurements of the carbon dioxide absorption by unilluminated leaves have demonstrated that all leaves so far examined, with the possible exception of leaves from the acid plant *Sedum praealtum*, absorb carbon dioxide in excess of that ascribable to the water they contain. Inasmuch as etiolated, yellow, and albino leaves exhibit as great an absorption as their chlorophyllous counterparts, it appears that chlorophyll is not the controlling factor in carbon dioxide absorption. This is confirmed by the fact that

the extracted leaf residues which still contain chlorophyll give no evidence of compound formation with carbon dioxide. Furthermore, chlorophyll and its magnesium-free derivative, pheophytin, both absorb carbon dioxide but show no indication of combining with it chemically.

By comparing the amount of carbon dioxide absorbed with that removed from the leaf by evacuation, it has been demonstrated that the absorption process is strictly reversible. This is true for killed as well as for living leaves.

The water in leaves absorbs carbon dioxide in proportion to the quantity of water present and in proportion to the partial pressure of the carbon dioxide.

Detailed analysis of absorption by sunflower leaves revealed that both the sap and the insoluble leaf residue absorb carbon dioxide. The absorption in the sap may be accounted for by the reaction of carbonic acid with the buffers present, chiefly with the phosphates. The absorption by the insoluble material is attributable to the alkaline-earth carbonates contained therein.

The author wishes to thank Professor A. C. Frazer of Cornell University for furnishing the seed from which the albino corn plants were grown. He is also indebted to Dr. H. A. Spoehr, who initiated the work in this laboratory on carbon dioxide absorption by unilluminated leaves, and to Dr. H. H. Strain for many helpful suggestions.

Division of Plant Biology
Carnegie Institution of Washington
Stanford University
Palo Alto, California

## LITERATURE CITED

1. Arens, K. Physiologisch polarisierter Massenaustausch und Photosynthese bei submersen Wasserpflanzen. II. Die $Ca(HCO_3)_2$-Assimilation. Jahrb. wiss. Bot. **83**: 513–560. 1936.
2. ————————. Manganablagerungen bei Wasserpflanzen als Folge des physiologisch polarisierten Massenaustausches. Protoplasma **30**: 104–129. 1938.
3. Bohr, Christian. Definition und Methode zur Bestimmung der Invasions- und Evasionscoefficienten bei der Auflösung von Gasen in Flüssigkeiten. Werthe der genannten Constanten sowie der Absorptionscoefficienten der Kohlensäure bei Auflösung in Wasser und in Chlornatriumlösungen. Ann. Phys. Chem. **68**: 500–525. 1899.
4. Blackman, F. F. Experimental researches on vegetable assimilation and respiration. II. On the paths of gaseous exchange between

aerial leaves and the atmosphere. Phil. Trans. Royal Soc., London **186B**: 503–562. 1895.

5. BROWN, HORACE T. The principles of diffusion, their analogies and applications. Jour. Chem. Soc. **113**: 559–585. 1918.
6. BROWN, HORACE T., and ESCOMBE, F. Static diffusion of gases and liquids in relation to the assimilation of carbon and translocation in plants. Phil. Trans. Royal Soc., London **193B**: 223–291. 1900.
7. DUNOYER, L. La Technique du Vide. Les Presses Univ. de France. Paris, 1924, pp. 15–21.
8. FIFE, J. M., and FRAMPTON, V. L. The effect of carbon dioxide upon the pH and certain nitrogen fractions of the sugar-beet plant. Jour. Biol. Chem. **109**: 643–655. 1935.
9. GUGGENHEIM, E. A., and SCHINDLER, T. D. Studies of cells with liquid-liquid junctions. V. Standards for hydrogen ion measurements and the second dissociation constant of phosphoric acid. Jour. Phys. Chem. **38**: 533–541. 1934.
10. ————, and ————. Salt effects on sulfonphthalein indicators. Jour. Phys. Chem. **38**: 543–556. 1934.
11. HARNED, HERBERT S., and EMBREE, NORRIS D. The temperature variation of ionization constants in aqueous solutions. Jour. Amer. Chem. Soc. **56**: 1050–1053. 1934.
12. HUBERT, B. The physical state of chlorophyll in the living plastid. Rec. trav. bot. néerl. **32**: 323–390. 1935.
13. KETELAAR, J. A. A., and HANSON, E. A. Elementary cell and space group of ethyl-chlorophyllide. Nature **140**: 196. 1937.
14. MACINNES, D. A., BELCHER, D., and SHEDLOVSKY, THEODORE. The meaning and standardization of the pH scale. Jour. Amer. Chem. Soc. **60**: 1094–1099. 1938.
15. MARTIN, S. H. The hydrion concentration of plant tissues. IV. The buffer of sunflower hypocotyl. Protoplasma **1**: 522–536. 1927.
16. NIMS, LESLIE FREDERICK. The second dissociation constant of phosphoric acid from 20° to 50°. Jour. Amer. Chem. Soc. **55**: 1946–1951. 1933.
17. PIRSON, A. Ernährungs- und stoffwechselphysiologische Untersuchungen an *Fontinalis* und *Chlorella*. Zeitschr. Bot. **31**: 193–267. 1937.
18. RABINOWITCH, E. Sorption of carbon dioxide by chlorophyll. Nature **141**: 39. 1938.
19. REILLY, J., RAE, W. N., and WHEELER, T. S. Physico-chemical methods, pp. 68–70. D. Van Nostrand Co., Inc., New York. 1927.
20. SEIDELL, ATHERTON. Solubilities of inorganic and organic compounds, Vol. I. p. 211. D. Van Nostrand Co., Inc., New York. 1919.

21. SHEDLOVSKY, THEODORE, and MACINNES, D. A. The first ionization constant of carbonic acid, 0° to 38°, from conductance measurements. Jour. Amer. Chem. Soc. **57**: 1705–1710. 1935.

22. SMITH, JAMES H. C. Carotene IV. The hydrogenation of carotenes obtained from different sources, of dihydrocarotene, and of lycopin. Jour. Biol. Chem. **96**: 35–51. 1932.

23. SPOEHR, H. A. Photosynthesis. Pp. 310–314. The Chemical Catalogue Co., New York. 1926.

24. ————, and MCGEE, J. M. Absorption of carbon dioxide the first step in photosynthesis. Science, n.s., **59**: 513–514. 1924.

25. ————, and ————. The absorption of $CO_2$ by the leaf material. Year Book, Carnegie Inst. Washington **22**: 53. 1924.

26. ————, and ————. Absorption of carbon dioxide by leaf material. Year Book, Carnegie Inst. Washington **23**: 132–133. 1924.

27. ————, and NEWTON, WILLIAM. Absorption of carbon dioxide by leaf material. Year Book, Carnegie Inst. Washington **24**: 160–161. 1925.

28. ————, and ————. Absorption of carbon dioxide by leaf material. Year Book, Carnegie Inst. Washington **25**: 177–180. 1926.

29. ————, SMITH, JAMES H. C., STRAIN, H. H., and MILNER, H. W. Absorption of carbon dioxide by the unilluminated leaf. *Cf.* Year Books, Carnegie Institution of Washington **35**: 201–204. 1936; **36**: 203–205, 1937; **37**: 213–215. 1938.

30. THORNTON, NORWOOD C. Carbon dioxide storage. IV. The influence of carbon dioxide on the acidity of plant tissue. Contrib. Boyce Thompson Inst. **5**: 403–418. 1933.

31. VAN SLYKE, DONALD D., and NEILL, JAMES M. The determination of gases in blood and other solutions by vacuum extraction and manometric measurement. I. Jour. Biol. Chem. **61**: 523–573. 1924. (*Cf.* VAN SLYKE, DONALD D., and SENDROY, JULIUS, JR., Jour. Biol. Chem. **73**: 127–144. 1927.)

32. WILLSTÄTTER, RICHARD, and STOLL, ARTHUR. Untersuchungen über Chlorophyll. Julius Springer, Berlin. 1913. a) Pp. 133–135; b) p. 269.

33. WILLSTÄTTER, RICHARD, and STOLL, ARTHUR. Untersuchungen über die Assimilation der Kohlensäure. Julius Springer, Berlin. 1918. a) pp. 172, 182; b) pp. 179, 192, 198, 217, 223; c) pp. 182, 183, 187; d) pp. 308, 292; e) p. 416; f) p. 436; g) pp. 298–305.

# DISTRIBUTION OF NITROGENOUS AND CARBOHYDRATE FRACTIONS AND OTHER SUBSTANCES IN EXPOSED AND COVERED PINEAPPLE SISTER SHOOTS[1]

C. P. Sideris, B. H. Krauss, and H. Y. Young

(WITH NINE FIGURES)

## Introduction

After the first fruit on a pineapple is removed, two or more lateral shoots frequently develop from the leaf axis of the mother plant and on these, fruits are borne the following year. It became of interest to study the difference in physiological behavior and chemical composition of such sister shoots, both on the same plant, grown in darkness and seasonal light, respectively, while receiving their entire inorganic nutrition and water supply by way of the roots and stem of the same mother plant. The studies reported below represent an attempt to furnish information on the behavior of pairs of sister shoots so treated. Another objective was to study the transfer of organic and inorganic solutes from the parent stem to the shoots, one grown in darkness and the other under daylight conditions.

Burkholder (**5**) has satisfactorily reviewed and discussed the literature on the rôle of light in the life of plants. As certain original sources of information bearing directly on this subject are mentioned, however, their data are compared and discussed in connection with the findings reported below. Brown and Trelease (**4**), working with *Cestrum nocturnum,* observed that the length of shoots of this plant decreased during the day, owing to water loss by transpiration. At night the shoots not only recovered their original length but also made further rapid growth. Trelease (**31**) observed a parallel case in *Musa sapientum.* Tolmachev (**29**) noted in Opuntia sp. that the rate of growth was synchronous with decreased acidity of the tissues. According to this investigator and Richards (**19**), the acidity of Opuntia sp. was greater during the night than during the day. Street (**27**) observed that *Pisum sativum,* grown in water cultures, absorbed more magnesium and calcium when exposed to long than to short periods of light. With respect to nitrate assimilation, Nightingale and Schermerhorn (**14**), observed in *Asparagus officinalis,* that nitrate was utilized very rapidly both in darkness and in light as long as the tissues were supplied with ample quantities of carbohydrates. Tottingham and Lease (**30**) observed, in the case of *Triticum sativum, Solanum lycopersicum,* and *Lycopersicum esculentum,* that, in blue to violet light, the percentage of protein increased; at the same time there was a decrease in

[1] Published with the approval of the Director as Technical Paper no. 116 of the Pineapple Experiment Station, University of Hawaii.

the amount of pentosans. The conditions favoring the synthesis or hydrolysis of proteins have been studied by different investigators. IVANOFF (**10**) observed that, for the synthesis of proteins in the absence of light, plants like *Brassica napus, Daucus carota,* and *Solanum tuberosum,* required a small amount of protein and a great supply of amides and readily assimilable carbohydrates. This, in general principle, is in agreement with the findings of most investigators. According to EISENMENGER (**7**), and KISHI and YOKOTA (**12**), the absence of light, causing a decrease in the supply of carbohydrates, favors the accumulation of nitrate in the plant and the hydrolysis of proteins; these processes result in an increase in the amino acid nitrogen content of the tissues. With insufficient quantities of light, where a normal rate of carbohydrate synthesis is limited, nitrogen cannot be used effectively by the plant, according to PRIESTLEY (**17**). Moreover, under conditions of prolonged darkness, proteins are apparently respired as the source of energy, according to NIGHTINGALE, *et al.* (**15**). PRIESTLEY and EWING (**18**) attribute the decreased rate of solute movement in the tissues of etiolated plants to structural modifications in the growing point of the plant. In pineapple plants grown in nutrient solutions, greater quantities of proteins and smaller quantities of soluble organic nitrogen were produced when the plants were exposed to light, than when they were kept in darkness, according to certain other studies of the authors (**24**).

## Experimental methods

During the period between the 2nd and 23rd of July, 1934, approximately two weeks after the fruits had been picked, 40 pairs of pineapple sister shoots were selected on the basis of one pair per mother plant. One of the members of each pair was covered with a bag made of light green oil cloth which fitted loosely around the shoot. The lower opening of the bag was partly closed to prevent the entrance of light. The other member of the pair was left exposed to light. These sister shoots will be designated as "covered" and "exposed," respectively.

At intervals of 17, 56, 77 and 105 days from the time that half the shoots were covered, samples consisting of from 5 to 10 pairs of shoots were detached from their mother plants. These shoots were segregated into covered and exposed groups and prepared for analysis. The technique described in a previous publication (**22**) was employed.

The leaves and stem were separated into homogeneous samples on the basis of age, morphological condition, and physiological function of the various sections of these organs. The chemical methods described in other publications (**21, 22**) were adopted.

It was of course impossible to detach the growing shoots and obtain their initial weights at the beginning of the experiments. Shoot weights for the

different groups, however, were obtained at the end of each experimental period. The various sections of the leaves and stem of the shoots removed at the end of all the four experimental periods were analyzed for moisture, chlorophyll, electrical resistance, titratable acidity, pH, nitrate nitrogen, reducing sugars and sucrose. The shoots removed at the end of the 56-day period were analyzed also for organic nitrogen fractions. The results from the various studies are presented in tables I to XI and in figures 2 to 9.

## Results

### Weights

The weights of the shoots from different plants varied somewhat, as far as we could determine by observation, at the time of selection although particular attention was paid to the uniformity of the pair of shoots on each mother plant. Because of inherent variability some pairs of shoots were smaller than other pairs, and the degree of variability from the mean weight was greater than 20 per cent. for the shoots removed at the end of the different experimental periods. Average shoot weights are presented in table I.

TABLE I

WEIGHT OF EXPOSED AND COVERED PINEAPPLE SHOOTS REMOVED FROM THEIR MOTHER PLANTS AT THE END OF VARIOUS PERIODS OF TIME

| Period | Weight of shoots | |
|---|---|---|
| | Exposed | Covered |
| *days* | *gm.* | *gm.* |
| 17 | 618 | 635 |
| 56 | 980 | 1010 |
| 77 | 1303 | 1110 |
| 105 | 1465 | 921 |

The data suggest that pineapple shoots kept in darkness by covering for a period of one to two months, may grow without any external symptoms of detriment when attached to the mother plant. Under the longer experimental periods, however, when the shoots were allowed to remain on the plants for 77 and 105 days, the lesser weights of the covered shoots represent a decreased rate of growth caused by the effects of shading. The losses in weight of the shoots during these two periods were accompanied also with more pronounced morphological and physico-chemical changes in the leaves and stem, as presented later.

### Moisture

The moisture content of the various sections of the leaves and of the stem of the covered and exposed shoots removed at the end of all the four experi-

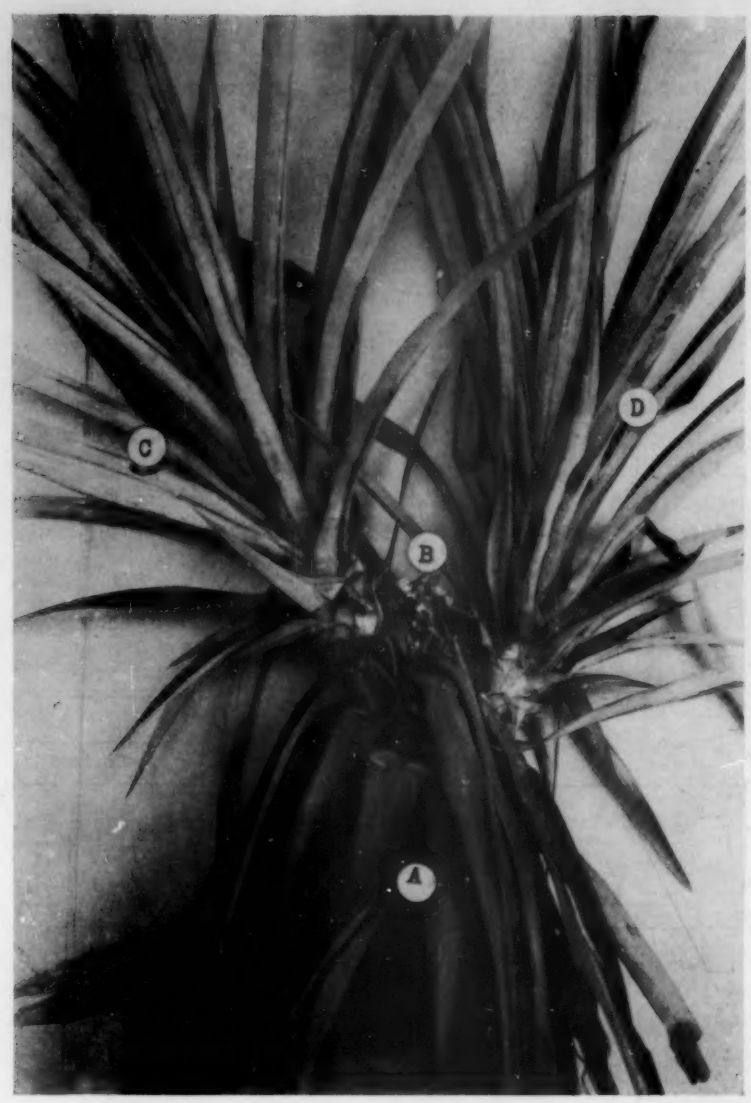



FIG. 1. Mother plant (A) with the peduncle (B) of the first season's fruit, and two shoots (C and D) which will produce the second season's fruit.

mental periods are presented in table II. Figure 2 illustrates the distribution of moisture in the sections of the exposed and covered shoots removed at the end of the 105-day period.

The data show, in general, that the moisture percentage of the tissues of the exposed shoots decreased with advancing age while that of comparable

## TABLE II

MOISTURE CONTENT OF VARIOUS SECTIONS OF THE LEAVES AND STEM OF EXPOSED AND COVERED PINEAPPLE SISTER SHOOTS ATTACHED TO THEIR MOTHER PLANT FOR DIFFERENT PERIODS OF TIME

| PLANT SECTIONS | PERIODS | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 17 DAYS | | 56 DAYS | | 77 DAYS | | 105 DAYS | |
| | EXPOSED | COVERED | EXPOSED | COVERED | EXPOSED | COVERED | EXPOSED | COVERED |
| Leaves: | % | % | % | % | % | % | % | % |
| Old: | | | | | | | | |
| B1 (base) | 86.4 | 87.3 | 87.0 | 86.8 | 85.7 | 87.6 | 87.5 | 90.1 |
| B2 | 85.3 | 85.3 | 83.2 | 83.0 | 84.9 | 85.6 | 86.0 | 88.2 |
| B3 | 84.7 | 86.7 | 83.4 | 84.7 | 84.1 | 85.4 | 85.0 | 87.1 |
| B4 (tip) | ..... | ..... | 82.1 | 81.3 | 82.3 | 76.5 | 81.5 | 29.6* |
| Mature: | | | | | | | | |
| C1 (base) | 92.4 | 92.5 | 89.7 | 90.2 | 89.9 | 89.8 | 90.7 | 91.8 |
| C2 | 88.4 | 90.3 | 85.8 | 87.0 | 86.5 | 87.2 | 88.1 | 89.8 |
| C3 | 85.0 | 89.2 | 83.1 | 89.3 | 84.7 | 89.0 | 85.4 | 90.4 |
| C4 | ..... | ..... | 82.0 | 89.0 | 84.2 | 88.3 | 82.7 | 90.0 |
| C5 (tip) | ..... | ..... | 82.3 | 88.2 | 81.1 | 85.4 | 78.4 | 81.2 |
| Active: | | | | | | | | |
| D1 (base) | ..... | ..... | 91.4 | 90.8 | 91.2 | 92.4 | 92.0 | 93.0 |
| D2 | ..... | ..... | 88.0 | 89.8 | 88.2 | 89.3 | 88.4 | 90.6 |
| D3 | ..... | ..... | 84.8 | 89.2 | 86.8 | 90.4 | 85.2 | 91.4 |
| D4 | ..... | ..... | 83.7 | 90.1 | 84.2 | 90.3 | 84.6 | 91.2 |
| D5 (tip) | ..... | ..... | 83.5 | 88.2 | 81.5 | 88.0 | 79.6 | 89.6 |
| Young: | | | | | | | | |
| E1 (base) | ..... | ..... | 91.4 | 93.6 | 92.1 | 92.2 | 92.0 | 92.6 |
| E2 | ..... | ..... | 87.8 | 88.8 | 88.1 | 90.0 | 88.3 | 89.6 |
| E3 | ..... | ..... | 87.0 | 88.4 | 88.4 | 90.4 | 86.0 | 90.2 |
| E4+5 (tip) | ..... | ..... | 84.5 | 88.4 | 84.1 | 89.6 | 83.5 | 89.6 |
| Stem: | | | | | | | | |
| Basal (cortex) | 75.5 | 79.6 | ..... | 86.4 | 85.8 | 86.6 | 89.0 | 89.2 |
| " (pith) | 76.1 | 77.8 | 78.9 | ..... | 82.2 | 85.0 | 85.2 | 90.3 |
| Apical (cortex) | 87.3 | 79.1 | 83.7 | 87.6 | 87.5 | 89.5 | 90.0 | 90.2 |
| " (pith) | 86.3 | 87.6 | 84.5 | 88.1 | 85.8 | 89.2 | 87.2 | 90.6 |

* Tissues dead and possibly dried.

tissues of the covered shoots increased. This condition indicates that the amounts of solids per unit of fresh weight were greater in the tissues of the exposed than in those of the covered shoots.

### CHLOROPHYLL

The chlorophyll content of the sections of leaves of both exposed and covered shoots removed at the end of all the four periods is presented in table III. Figure 3 reports only the chlorophyll content of the leaf sections of covered and exposed shoots removed at the end of the 56-day period.

The data in table III show that the amounts of chlorophyll in the leaves

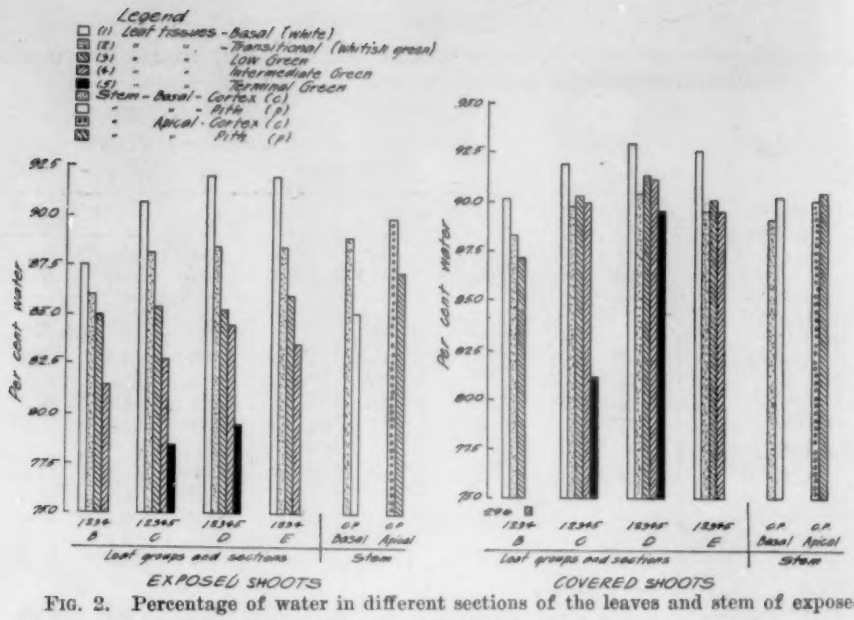

Legend
(1) Leaf tissues - Basal (white)
(2) " " - Transitional (whitish green)
(3) " " Low Green
(4) " " Intermediate Green
(5) " " Terminal Green
Stem - Basal - Cortex (c)
" " - Pith (p)
" Apical - Cortex (c)
" " Pith (p)
Per cent water
Leaf groups and sections
Stem
EXPOSED SHOOTS
COVERED SHOOTS

FIG. 2. Percentage of water in different sections of the leaves and stem of exposed and covered pineapple shoots at the end of the 105-day period.

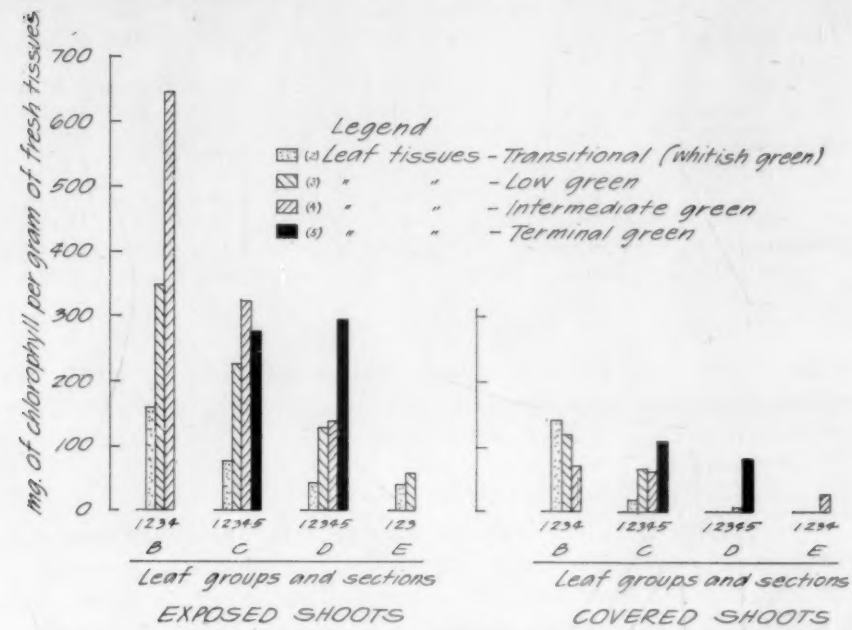

mg. of chlorophyll per gram of fresh tissues
Legend
(2) Leaf tissues - Transitional (whitish green)
(3) " " - Low green
(4) " " - Intermediate green
(5) " " - Terminal green
Leaf groups and sections
EXPOSED SHOOTS
COVERED SHOOTS

FIG. 3. Milligrams of chlorophyll per gram of fresh weight in different sections of the leaves of exposed and covered pineapple shoots at the end of the 56-day period.

## TABLE III

MILLIGRAMS OF CHLOROPHYLL PER GRAM OF FRESH WEIGHT IN VARIOUS SECTIONS OF THE LEAVES OF EXPOSED AND COVERED PINEAPPLE SISTER SHOOTS ATTACHED TO THE MOTHER PLANT FOR DIFFERENT PERIODS OF TIME

| Plant sections | Periods | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 17 days | | 56 days | | 77 days | | 105 days | |
| | Exposed | Covered | Exposed | Covered | Exposed | Covered | Exposed | Covered |
| | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* |
| Leaves: | | | | | | | | |
| Old: | | | | | | | | |
| B1 (base) | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| B2 | 0.122 | 0.150 | 0.162 | 0.148 | ..... | 0.040 | 0.124 | ..... |
| B3 | 0.553 | 0.518 | 0.346 | 0.126 | 0.388 | 0.149 | 0.299 | 0.038 |
| B4 (tip) | ..... | ..... | 0.718 | 0.072 | 0.390 | 0.306 | 0.592 | 0.000 |
| Mature: | | | | | | | | |
| C1 (base) | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| C2 | 0.131 | 0.050 | 0.071 | 0.017 | ..... | 0.000 | 0.100 | 0.000 |
| C3 | 0.297 | 0.232 | 0.220 | 0.078 | 0.258 | 0.000 | 0.346 | 0.000 |
| C4 | ..... | ..... | 0.320 | 0.075 | 0.396 | 0.093 | 0.441 | ..... |
| C5 (tip) | ..... | ..... | 0.272 | 0.102 | 0.290 | 0.257 | 0.557 | ..... |
| Active: | | | | | | | | |
| D1 (base) | ..... | ..... | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| D2 | ..... | ..... | 0.035 | 0.000 | 0.073 | 0.000 | 0.075 | 0.000 |
| D3 | ..... | ..... | 0.126 | 0.000 | 0.203 | 0.000 | 0.206 | 0.000 |
| D4 | ..... | ..... | 0.128 | 0.007 | ..... | 0.000 | 0.340 | 0.000 |
| D5 (tip) | ..... | ..... | 0.294 | 0.082 | 0.287 | 0.160 | 0.478 | ..... |
| Young: | | | | | | | | |
| E1 (base) | ..... | ..... | 0.000 | 0.000 | 0.000 | ..... | 0.000 | 0.000 |
| E2 | ..... | ..... | 0.038 | 0.000 | ..... | ..... | 0.041 | 0.000 |
| E3 | ..... | ..... | 0.048 | 0.000 | 0.075 | ..... | 0.162 | 0.000 |
| E4+5 (tip) | ..... | ..... | ..... | 0.020 | 0.400 | ..... | 0.301 | 0.000 |

of the covered plants decreased with prolonged darkness; *i.e.*, during a period of 105 days. The low chlorophyll values for the covered shoots during the various periods resulted chiefly from the inability of the plant to produce this substance in the new leaves formed after covering, and also to some losses caused by the breaking down of chlorophyll in the older leaves. The terminal sections of the leaves of the covered shoots removed at the end of the 56-day period contained less amounts of chlorophyll than the same sections of leaves from covered shoots removed at the end of the 77-day period. The greater amounts in the shoots removed at the end of the latter period may indicate variations in the initial chlorophyll content of the experimental material, a state which occurs even under similar nutritional and environmental conditions. It is safe to assume, therefore, that after a very prolonged period of darkness, such as 105 days, the chlorophyll of pineapple leaves undergoes from partial to complete decomposition.

### Electrical resistance

The electrical resistance of the extracted sap was determined with a set-up of standard Leeds and Northrup instruments consisting of a student potentiometer, a 6-dial resistance box, an a. c. pointer galvanometer and a Henry-type conductivity cell. The results obtained for the shoots removed at the end of the four different periods are presented in table IV. Figure 4 reports only the data for the shoots removed at the end of the 56-day period.

The data in the table show that the electrical resistance of the sap of the tissues in the covered shoots increased with longer periods of darkness. The electrical resistance values of the terminal sections of the leaves were influenced more by the effects of darkness than the basal sections. The sections

## TABLE IV

ELECTRICAL RESISTANCE OF THE EXTRACTED SAP OF VARIOUS LEAF AND STEM SECTIONS OF EXPOSED AND COVERED PINEAPPLE SISTER SHOOTS ATTACHED TO THEIR MOTHER PLANT FOR DIFFERENT PERIODS OF TIME

| Plant sections | Periods | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 17 days | | 56 days | | 77 days | | 105 days | |
| | Exposed | Covered | Exposed | Covered | Exposed | Covered | Exposed | Covered |
| | *ohms* | *ohms* | *ohms* | *ohms* | *ohms* | *ohms* | *ohms* | *ohms* |
| Leaves: | | | | | | | | |
| Old: | | | | | | | | |
| B1 (base) | 344 | 350 | 167 | 182 | 116 | ..... | 116 | 112 |
| B2 | 332 | 324 | 157 | 182 | 109 | ..... | 95 | 145 |
| B3 | 220 | 280 | 117 | 200 | 73 | ..... | 87 | 203 |
| B4 (tip) | ..... | ..... | 73 | 157 | 72 | ..... | 65 | ..... |
| Mature: | | | | | | | | |
| C1 (base) | 262 | 320 | 100 | 147 | 87 | ..... | 109 | 87 |
| C2 | 294 | 312 | 117 | 178 | 99 | ..... | 95 | 138 |
| C3 | 240 | 292 | 105 | 178 | 69 | ..... | 73 | 124 |
| C4 | ..... | ..... | 88 | 160 | 60 | ..... | 61 | 137 |
| C5 (tip) | ..... | ..... | 75 | 146 | 47 | ..... | 52 | 119 |
| Active: | | | | | | | | |
| D1 (base) | ..... | ..... | 88 | 186 | 50 | ..... | 93 | 105 |
| D2 | ..... | ..... | 109 | 182 | 85 | ..... | 102 | 115 |
| D3 | ..... | ..... | 134 | 178 | 87 | ..... | 77 | 145 |
| D4 | ..... | ..... | 134 | 178 | 61 | ..... | 58 | 122 |
| D5 (tip) | ..... | ..... | 91 | 109 | 52 | ..... | 51 | 113 |
| Young: | | | | | | | | |
| E1 (base) | ..... | ..... | 97 | 112 | 67 | ..... | 95 | 87 |
| E2 | ..... | ..... | 145 | 156 | ..... | ..... | 122 | 160 |
| E3 | ..... | ..... | 128 | 165 | 102 | ..... | 128 | 131 |
| E4+5 (tip) | ..... | ..... | ..... | 195 | 87 | ..... | 68 | 135 |
| Stem: | | | | | | | | |
| Basal (cortex) | 260 | 240 | 102 | 93 | 57 | ..... | 63 | 73 |
| " (pith) | 218 | 206 | 88 | 78 | 46 | ..... | 50 | 48 |
| Apical (cortex) | ..... | ..... | 80 | 78 | 58 | ..... | 60 | 53 |
| " (pith) | ..... | ..... | 60 | 70 | 47 | ..... | 48 | 58 |

of the stem of both covered and exposed shoots do not show as great contrasts as do those of the leaves.

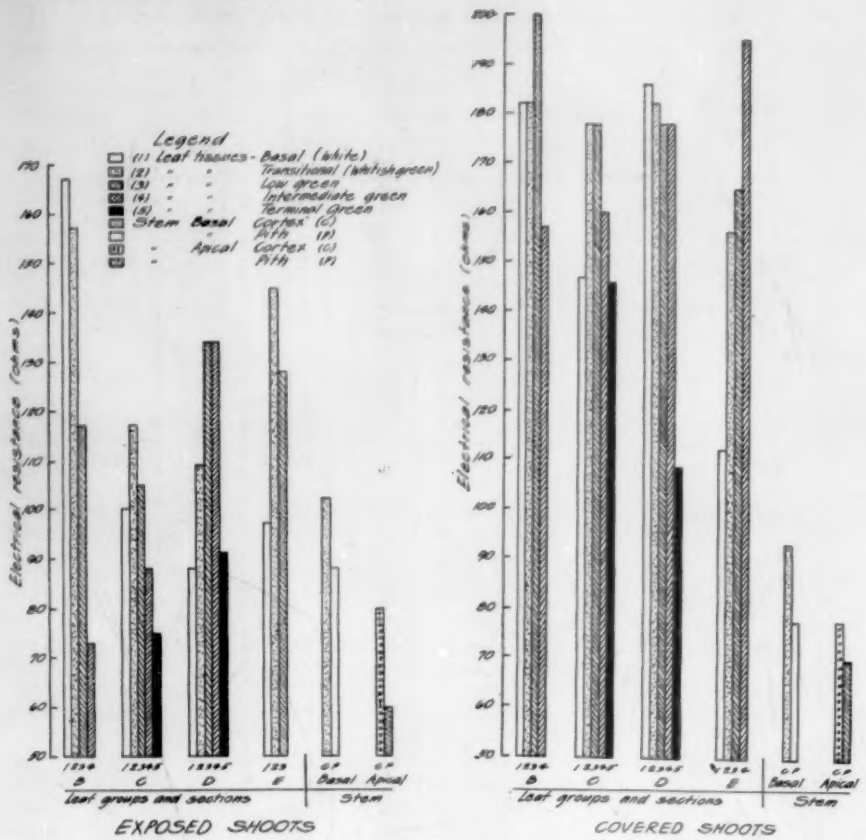



FIG. 4. Electrical resistance (ohms) of the extracted sap of different sections of the leaves and stem of exposed and covered pineapple shoots at the end of the 56-day period.

## ACIDITY

Determinations of titratable acidity were made on aliquots from aqueous solutions of the extracted sap from 10-gram samples of fresh tissues. A different aliquot from the same aqueous extract was used for the colorimetric determination of the pH values. The data for titratable acidity for the covered and exposed shoots, removed at the end of all four periods, are presented in table V and in figure 5. The values for pH are reported in table VI and figure 6.

Titratable acidity, reported as percentage of citric acid, increased in the chlorophyllous sections of the exposed shoots with advancing age, while it

## TABLE V

TITRATABLE ACIDITY AS CITRIC ACID ON A FRESH WEIGHT BASIS IN VARIOUS SECTIONS OF THE LEAVES AND STEM OF EXPOSED AND COVERED PINEAPPLE SISTER SHOOTS ATTACHED TO THEIR MOTHER PLANT FOR DIFFERENT PERIODS OF TIME

| PLANT SECTIONS | PERIODS | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 17 DAYS | | 56 DAYS | | 77 DAYS | | 105 DAYS | |
| | EXPOSED | COVERED | EXPOSED | COVERED | EXPOSED | COVERED | EXPOSED | COVERED |
| Leaves: | % | % | % | % | % | % | % | % |
| Old: | | | | | | | | |
| B1 (base) | 0.192 | 0.192 | 0.038 | 0.093 | 0.064 | ..... | 0.045 | 0.090 |
| B2 | 0.179 | 0.179 | 0.051 | 0.064 | 0.160 | ..... | 0.154 | 0.025 |
| B3 | 1.536 | 0.486 | 0.435 | 0.151 | 0.864 | ..... | 0.813 | 0.064 |
| B4 (tip) | ..... | ..... | 0.832 | 0.333 | 0.960 | ..... | 1.197 | ..... |
| Mature: | | | | | | | | |
| C1 (base) | 0.224 | 0.179 | 0.179 | 0.218 | 0.160 | ..... | 0.090 | 0.173 |
| C2 | 0.243 | 0.154 | 0.102 | 0.090 | 0.060 | ..... | 0.190 | 0.090 |
| C3 | 1.677 | 0.333 | 0.525 | 0.064 | 0.960 | ..... | 1.114 | 0.090 |
| C4 | ..... | ..... | 1.254 | 0.640 | 1.824 | ..... | 1.818 | 0.173 |
| C5 (tip) | ..... | ..... | 1.472 | 0.256 | 2.110 | ..... | 1.920 | 0.282 |
| Active: | | | | | | | | |
| D1 (base) | ..... | ..... | 0.179 | 0.100 | 0.320 | ..... | 0.128 | 0.154 |
| D2 | ..... | ..... | 0.128 | 0.128 | 0.160 | ..... | 0.109 | 0.064 |
| D3 | ..... | ..... | 0.540 | 0.512 | 0.448 | ..... | 0.813 | 0.090 |
| D4 | ..... | ..... | 1.090 | 0.461 | 1.660 | ..... | 1.837 | 0.154 |
| D5 (tip) | ..... | ..... | 1.357 | 0.320 | 2.050 | ..... | 2.138 | 0.282 |
| Young: | | | | | | | | |
| E1 (base) | ..... | ..... | 0.154 | 0.448 | 0.224 | ..... | 0.090 | 0.128 |
| E2 | ..... | ..... | 0.102 | 0.077 | ..... | ..... | 0.109 | 0.045 |
| E3 | ..... | ..... | 0.435 | 0.090 | 0.160 | ..... | 0.538 | 0.064 |
| E4 + 5 (tip) | ..... | ..... | ..... | 0.102 | 0.960 | ..... | 1.709 | 0.128 |
| Stem: | | | | | | | | |
| Basal (cortex) | 0.333 | 0.352 | 0.179 | 0.256 | 0.224 | ..... | 0.154 | 0.256 |
| " (pith) | 0.435 | 0.422 | 0.192 | 0.358 | 0.320 | ..... | 0.192 | 0.301 |
| Apical (cortex) | ..... | ..... | 0.256 | 0.550 | 0.320 | ..... | 0.256 | 0.429 |
| " (pith) | ..... | ..... | 0.282 | 0.717 | 0.480 | ..... | 0.256 | 0.384 |

decreased in comparable leaf sections of the covered shoots, as shown in table V. The stem and the non-chlorophyllous basal section of the leaves of the covered shoots contained greater amounts of titratable acidity than those of the exposed shoots.

The values for pH presented in table VI and in figure 6 follow more or less the course of titratable acidity. They are high in the basal non-chlorophyllous and low in the chlorophyllous sections of the leaves of the exposed shoots, while in the covered shoots the pH values of the different leaf sections approach a constant level as the total time of shading increased. If we contrast the curves for the pH values of comparable sections of the leaves of exposed and covered shoots we find that in the former the values decrease from the basal to the apical sections, while in the latter they fluctuate within narrow limits.

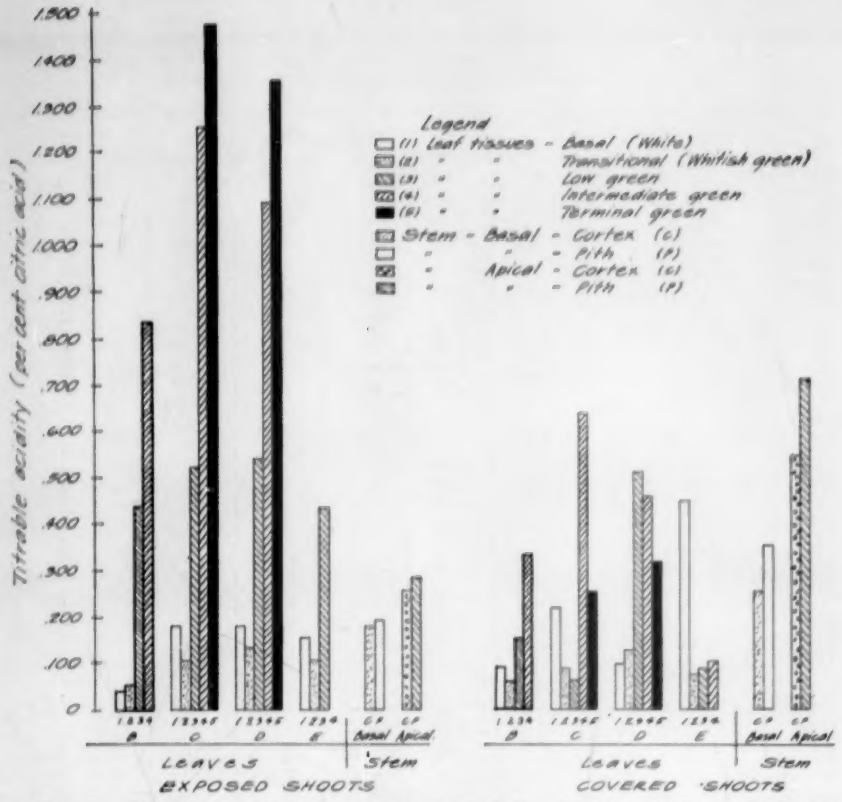



FIG. 5. Titratable acidity as percentage of citric acid on a fresh weight basis of different sections of the leaves and stem of exposed and covered pineapple shoots at the end of the 56-day period.

## DISTRIBUTION OF NITROGENOUS FRACTIONS

The distribution of nitrate in the various sections of the leaves of the exposed and covered lots of shoots, removed at the end of all the four periods, are reported in table VII. Table VIII contains the analytical data for the fractions of inorganic, soluble organic, and protein nitrogen in the different sections of the leaves and stem of the exposed and covered shoots removed at the end of the 56-day period. The data in table VIII are presented in graphic form in figures 7 and 8.

The values in table VII show, in general, that the movement of nitrate from the mother plant to the attached sister shoots was greater for the exposed than for the covered shoots. In practically all cases, the amounts of nitrate in the non-chlorophyllous sections of the mature C, active D, and young E groups of leaves were greater for the exposed than for the covered

## TABLE VI

HYDROGEN-ION CONCENTRATION OF THE EXTRACTED SAP OF VARIOUS SECTIONS OF THE LEAVES AND STEM OF EXPOSED AND COVERED PINEAPPLE SISTER SHOOTS ATTACHED TO THEIR MOTHER PLANT FOR DIFFERENT PERIODS OF TIME

| PLANT SECTIONS | PERIODS | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 17 DAYS | | 56 DAYS | | 77 DAYS | | 105 DAYS | |
| | EXPOSED | COVERED | EXPOSED | COVERED | EXPOSED | COVERED | EXPOSED | COVERED |
| Leaves: | *pH* | *pH* | *pH* | *pH* | *pH* | *pH* | *pH* | *pH* |
| Old: | | | | | | | | |
| B1 (base) | 5.4 | 5.5 | 4.9 | 3.8 | 5.6 | ..... | 5.0 | 5.0 |
| B2 | 4.7 | 5.5 | 5.2 | 4.8 | 5.0 | ..... | 4.8 | 5.0 |
| B3 | 4.0 | 5.1 | 4.3 | 3.8 | 4.2 | ..... | 4.0 | 4.8 |
| B4 (tip) | ..... | ..... | 4.2 | 4.6 | 4.2 | ..... | 4.2 | ..... |
| Mature: | | | | | | | | |
| C1 (base) | 5.6 | 5.7 | 5.4 | 5.0 | 5.0 | ..... | 5.0 | 5.0 |
| C2 | 4.8 | 5.7 | 5.0 | 5.1 | 5.0 | ..... | 4.6 | 5.2 |
| C3 | 3.9 | 5.0 | 4.0 | 5.5 | 4.2 | ..... | 4.2 | 5.2 |
| C4 | ..... | ..... | 3.6 | 3.8 | 3.8 | ..... | 4.0 | 5.2 |
| C5 (tip) | ..... | ..... | 3.7 | 5.2 | 4.0 | ..... | 4.0 | 5.2 |
| Active: | | | | | | | | |
| D1 (base) | ..... | ..... | 5.5 | 5.4 | 5.2 | ..... | 5.2 | 5.3 |
| D2 | ..... | ..... | 5.4 | 4.8 | 5.2 | ..... | 5.0 | 5.6 |
| D3 | ..... | ..... | 4.4 | 3.6 | 4.4 | ..... | 4.2 | 5.2 |
| D4 | ..... | ..... | 3.8 | 4.3 | 3.7 | ..... | 3.8 | 5.2 |
| D5 (tip) | ..... | ..... | 3.6 | 5.0 | 3.7 | ..... | 3.9 | 5.2 |
| Young: | | | | | | | | |
| E1 (base) | ..... | ..... | 5.6 | 4.1 | 5.4 | ..... | 5.5 | 5.5 |
| E2 | ..... | ..... | 5.4 | 5.6 | ..... | ..... | 4.8 | 5.4 |
| E3 | ..... | ..... | 4.2 | 5.4 | 4.7 | ..... | 4.2 | 5.3 |
| E4 + 5 (tip) | ..... | ..... | ..... | 5.4 | 4.0 | ..... | 3.8 | 5.2 |
| Stem: | | | | | | | | |
| Basal (cortex) | 5.6 | 5.3 | 4.8 | 4.8 | 5.0 | ..... | 5.0 | 5.0 |
| " (pith) | 5.5 | 5.5 | 5.1 | 5.0 | 5.0 | ..... | 5.2 | 5.0 |
| Apical (cortex) | ..... | ..... | 5.2 | 5.0 | 5.2 | ..... | 5.2 | 5.0 |
| " (pith) | ..... | ..... | 5.4 | 4.8 | 5.2 | ..... | 5.2 | 5.2 |

shoots. Exceptions may be found in the basal sections of the senile B group of leaves, and in the stem. The rate of nitrate assimilation, as indicated by the difference between the amounts of this substance in the basal non-chlorophyllous sections (no. 1) and in the chlorophyllous sections (no. 3 or 4) of the C, D, and E groups of leaves, is considerably greater for the exposed than for the covered shoots. The causes for the low rate of nitrate assimilation in the leaves of the covered shoots may be attributed to the small amounts of chlorophyll and carbohydrate in the leaves and possibly unknown substances.

Certain differences that may be observed in the amounts of nitrate in the non-chlorophyllous sections of both covered and exposed lots of sister shoots at the end of the different experiment periods were caused by the length of

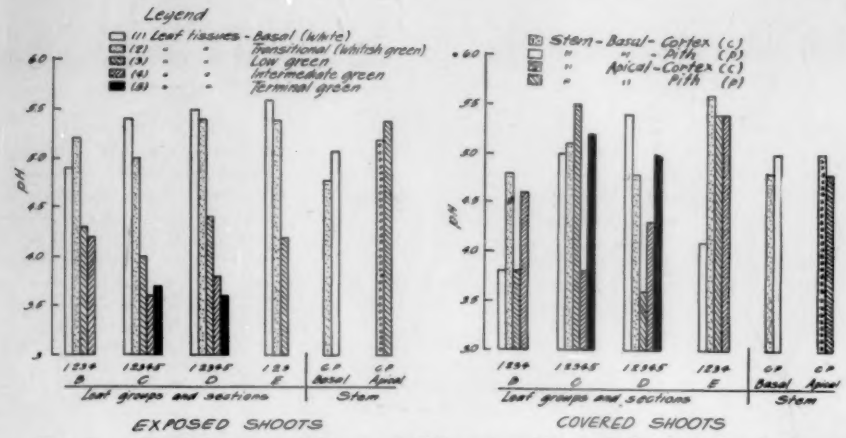



FIG. 6. pH of the extracted sap of different sections of the leaves and stem of exposed and covered pineapple shoots at the end of the 56-day period.

## TABLE VII

MILLIGRAMS OF NITRATE NITROGEN PER GRAM OF FRESH WEIGHT IN VARIOUS SECTIONS OF THE LEAVES AND STEM OF EXPOSED AND COVERED PINEAPPLE SISTER SHOOTS ATTACHED TO THEIR MOTHER PLANT FOR DIFFERENT PERIODS OF TIME

| PLANT SECTIONS | PERIODS | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 17 DAYS | | 56 DAYS | | 77 DAYS | | 105 DAYS | |
| | EXPOSED | COVERED | EXPOSED | COVERED | EXPOSED | COVERED | EXPOSED | COVERED |
| | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* |
| Leaves: | | | | | | | | |
| Old: | | | | | | | | |
| B1 (base) | 0.310 | 0.185 | 0.357 | 0.292 | 0.194 | 0.169 | 0.495 | 0.520 |
| B2 | 0.231 | 0.174 | 0.237 | 0.184 | 0.060 | 0.068 | 0.316 | 0.433 |
| B3 | 0.007 | 0.000 | 0.031 | 0.036 | 0.012 | 0.044 | 0.019 | 0.163 |
| B4 (tip) | ..... | ..... | 0.008 | 0.000 | 0.007 | 0.019 | 0.004 | ..... |
| Mature: | | | | | | | | |
| C1 (base) | 0.338 | 0.105 | 0.394 | 0.109 | 0.427 | 0.066 | 0.672 | 0.163 |
| C2 | 0.091 | 0.105 | 0.356 | 0.112 | 0.257 | 0.072 | 0.427 | 0.178 |
| C3 | 0.003 | 0.025 | 0.025 | 0.106 | 0.014 | 0.058 | 0.012 | 0.150 |
| C4 | ..... | ..... | 0.008 | 0.042 | 0.000 | 0.057 | 0.002 | 0.116 |
| C5 (tip) | ..... | ..... | 0.000 | 0.006 | 0.000 | 0.022 | 0.000 | 0.079 |
| Active: | | | | | | | | |
| D1 (base) | ..... | ..... | 0.465 | 0.069 | 0.267 | 0.058 | 0.642 | 0.061 |
| D2 | ..... | ..... | 0.310 | 0.056 | 0.267 | 0.023 | 0.427 | 0.037 |
| D3 | ..... | ..... | 0.089 | 0.032 | 0.044 | 0.031 | 0.020 | 0.024 |
| D4 | ..... | ..... | 0.021 | 0.029 | 0.000 | 0.017 | 0.002 | 0.021 |
| D5 (tip) | ..... | ..... | 0.000 | 0.026 | 0.000 | 0.023 | 0.000 | 0.026 |
| Young: | | | | | | | | |
| E1 (base) | ..... | ..... | 0.205 | 0.080 | 0.151 | 0.039 | 0.357 | 0.046 |
| E2 | ..... | ..... | 0.033 | 0.042 | 0.116 | 0.028 | 0.171 | 0.033 |
| E3 | ..... | ..... | 0.019 | 0.026 | 0.060 | 0.025 | 0.015 | 0.021 |
| E4 + 5 (tip) | ..... | ..... | 0.000 | 0.025 | 0.000 | 0.028 | 0.005 | 0.022 |
| Stem: | | | | | | | | |
| Basal (cortex) | 0.149 | 0.185 | 0.347 | 0.530 | 0.538 | 0.237 | 0.608 | 0.588 |
| " (pith) | 0.268 | 0.143 | 0.131 | 0.440 | 0.567 | 0.243 | 0.924 | 0.588 |
| Apical (cortex) | 0.350 | 0.366 | 0.356 | 0.257 | 0.231 | 0.170 | 0.463 | 0.562 |
| " (pith) | 0.535 | 0.544 | 0.144 | 0.300 | 0.280 | 0.200 | 0.602 | 0.575 |

## TABLE VIII

MILLIGRAMS PER GRAM OF FRESH WEIGHT OF INORGANIC, SOLUBLE ORGANIC, AND PROTEIN NITROGEN FRACTIONS IN DIFFERENT SECTIONS OF THE LEAVES AND STEM OF EXPOSED AND COVERED PINEAPPLE SISTER SHOOTS ATTACHED TO THEIR MOTHER PLANT FOR 56 DAYS

| PLANT SECTIONS | MILLIGRAMS OF NITROGEN PER GRAM OF FRESH TISSUE | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | EXPOSED SHOOTS | | | | | | | COVERED SHOOTS | | | | | | |
| | INORGANIC | | SOLUBLE ORGANIC | | | | PROTEIN | INORGANIC | | SOLUBLE ORGANIC | | | | PROTEIN |
| | AMMONIUM* | NITRATE | AMIDE | ALPHA-AMINO | REST SOLUBLE | TOTAL | | AMMONIUM | NITRATE | AMIDE | ALPHA-AMINO | REST SOLUBLE | TOTAL | |
| Leaves: | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* |
| Old: | | | | | | | | | | | | | | |
| B1 (base) | 0.0056 | 0.357 | ..... | 0.274 | 0.370 | ..... | 0.325 | 0.003 | 0.292 | 0.040 | 0.270 | 0.200 | 0.510 | 0.168 |
| B2 | 0.0029 | 0.237 | 0.014 | 0.295 | 0.195 | 0.504 | 0.420 | 0.014 | 0.184 | 0.014 | 0.227 | 0.165 | 0.406 | 0.112 |
| B3 | 0.0067 | 0.031 | 0.017 | 0.405 | 0.559 | 0.981 | 0.840 | 0.027 | 0.036 | 0.030 | 0.392 | 0.295 | 0.717 | 0.428 |
| B4 (tip) | ..... | 0.008 | 0.235 | 0.890 | 1.180 | 2.305 | 1.240 | 0.065 | 0.000 | 0.060 | 0.567 | 0.633 | 1.260 | 0.614 |
| Mature: | | | | | | | | | | | | | | |
| C1 (base) | 0.0052 | 0.394 | 0.070 | 0.340 | 0.660 | 1.070 | 0.368 | 0.065 | 0.109 | 0.067 | 0.480 | 0.360 | 0.907 | 0.245 |
| C2 | 0.0070 | 0.356 | 0.056 | 0.406 | 0.266 | 0.728 | 0.502 | 0.046 | 0.112 | 0.032 | 0.338 | 0.124 | 0.494 | 0.350 |
| C3 | 0.0029 | 0.025 | 0.056 | 0.548 | 0.438 | 1.042 | 0.646 | 0.050 | 0.106 | 0.033 | 0.468 | 0.280 | 0.781 | 0.394 |
| C4 | ..... | 0.008 | 0.112 | 0.736 | 0.748 | 1.596 | 0.870 | 0.035 | 0.042 | 0.034 | 0.459 | 0.381 | 0.874 | 0.516 |
| C5 (tip) | ..... | 0.000 | 0.168 | 1.020 | 1.480 | 2.668 | 0.870 | 0.075 | 0.006 | 0.018 | 0.540 | 0.860 | 1.418 | 0.525 |
| Active: | | | | | | | | | | | | | | |
| D1 (base) | ..... | 0.465 | ..... | 0.684 | 0.519 | ..... | 0.313 | 0.022 | 0.069 | 0.040 | 0.503 | 0.487 | 1.030 | 0.327 |
| D2 | ..... | 0.310 | ..... | 0.548 | 0.292 | ..... | 0.340 | 0.011 | 0.056 | 0.018 | 0.432 | 0.128 | 0.578 | 0.310 |
| D3 | ..... | 0.089 | ..... | 0.428 | 0.272 | ..... | 0.438 | 0.012 | 0.032 | 0.046 | 0.393 | 0.167 | 0.606 | 0.348 |
| D4 | ..... | 0.021 | ..... | 0.428 | 0.412 | ..... | 0.682 | 0.041 | 0.029 | 0.092 | 0.439 | 0.275 | 0.806 | 0.464 |
| D5 (tip) | ..... | 0.000 | ..... | 0.570 | 0.810 | ..... | 0.885 | 0.023 | 0.026 | 0.134 | 0.540 | 0.650 | 1.324 | ..... |
| Young: | | | | | | | | | | | | | | |
| E1 (base) | ..... | 0.205 | ..... | 1.080 | 0.404 | ..... | 0.400 | 0.003 | 0.080 | 0.119 | 0.810 | 0.670 | 1.599 | ..... |
| E2 | ..... | 0.033 | ..... | 0.970 | 0.332 | ..... | 0.317 | 0.019 | 0.042 | 0.046 | 0.548 | 0.224 | 0.818 | ..... |
| E3 | ..... | 0.019 | ..... | 0.658 | 0.274 | ..... | 0.514 | 0.012 | 0.026 | 0.046 | 0.720 | ..... | ..... | 0.347 |
| E4+5 (tip) | ..... | 0.000 | ..... | ..... | ..... | ..... | ..... | 0.012 | 0.026 | 0.046 | 0.470 | 0.090 | 0.606 | 0.206 |
| Stem: | | | | | | | | | | | | | | |
| Basal (cortex) | ..... | 0.347 | ..... | 1.190 | 0.070 | ..... | 1.225 | 0.011 | 0.530 | 0.080 | 0.712 | 0.110 | 0.902 | ..... |
| " (pith) | ..... | 0.131 | ..... | 0.822 | 1.978 | ..... | 1.960 | 0.014 | 0.440 | 0.050 | 0.674 | 0.901 | 1.625 | 0.467 |
| Apical (cortex) | ..... | 0.356 | 0.028 | 0.862 | 0.351 | 1.241 | 0.850 | 0.023 | 0.257 | 0.059 | 0.988 | 1.212 | 2.259 | 0.805 |
| " (pith) | ..... | 0.144 | ..... | 0.988 | 0.992 | ..... | 1.060 | 0.050 | 0.300 | 0.077 | 1.280 | 0.905 | 2.262 | 0.710 |

* Data obtained from a different exposed shoot.

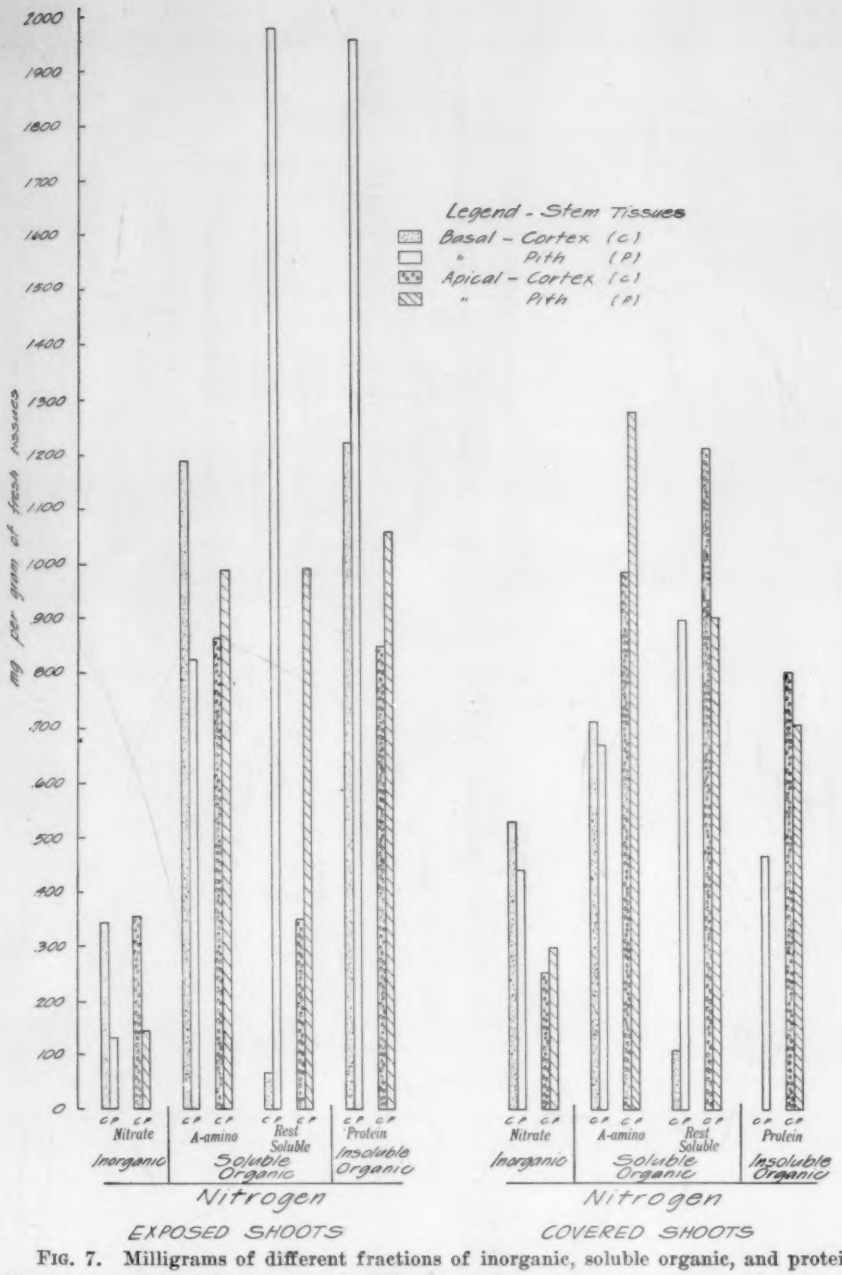



FIG. 7. Milligrams of different fractions of inorganic, soluble organic, and protein nitrogen per gram of fresh weight in different sections of the stem of exposed and covered pineapple shoots at the end of the 56-day period.

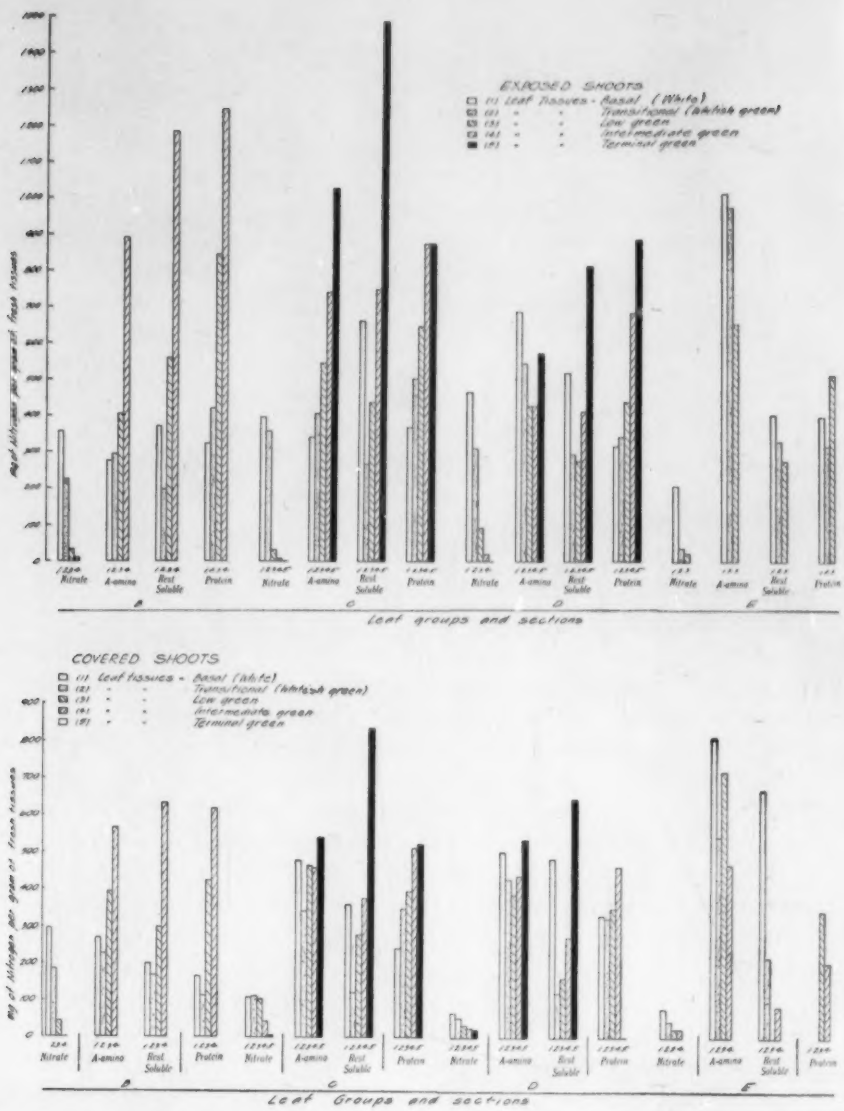



FIG. 8. Milligrams of different fractions of inorganic, soluble organic, and protein nitrogen per gram of fresh weight in different sections of the leaves of groups B, C, D, and E of exposed (A) and covered (B) pineapple shoots at the end of the 56-day period.

the interval between applications of fertilizer salts and time of harvesting. The results show, in general, that in pineapple leaves devoid of chlorophyll, nitrate is not reduced and converted to organic nitrogen compounds at the same rate that it is reduced and converted in chlorophyllous leaves. With

respect to the distribution of inorganic and organic nitrogen in the exposed and covered shoots removed at the end of the 56-day period, the data in table VIII show the following conditions:

1. Ammonium was present in relatively small amounts in the various sections of the leaves and stem of the covered and exposed shoots. The distribution of nitrate in the two lots of shoots and the conditions favoring or inhibiting its assimilation have been discussed.

2. The amounts of soluble amide nitrogen (glutamine and asparagine) increased from the transitional subchlorophyllous (no. 2) section to the terminal sections of the leaves of groups B and C of the exposed shoots. In the covered shoots this trend was not well pronounced. The basal nonchlorophyllous sections contained relatively larger amounts of amide nitrogen than the transitional sections immediately above. The quantities of amide nitrogen in the D group of leaves of the covered shoots increased from the transitional (no. 2) to the terminal (no. 5) section. In the E group of leaves of the covered shoots the amounts of amide nitrogen were relatively great in the basal section, while in the transitional and chlorophyllous sections they were appreciably smaller.

There are no data for the amide nitrogen content of the leaf groups D and E of the exposed plants because of the accidental loss of samples.

3. Alpha-amino nitrogen was relatively very abundant in most sections of the leaves and of the stem. Its distribution in the mature leaves of exposed shoots increased from the basal (no. 1) to the terminal (nos. 4 and 5) sections. In comparable sections of the leaves of the covered shoots, the trend of this distribution was irregular. In the active D and in the young E groups of leaves of the exposed shoots, where production of new tissues had not reached completion, more alpha-amino nitrogen accumulated in the basal than in the terminal sections. In the D and E groups of leaves of the covered shoots the trend of alpha-amino nitrogen distribution was practically the same as in the exposed shoots, excepting that the quantities were relatively smaller. The amounts of alpha-amino nitrogen in the cortex of the mature and aged basal section of the stem of the exposed shoots were greater than those in the cortex of the young and growing apical section, indicating that alpha-amino nitrogen was utilized more rapidly in the growing than in the mature tissues of the stem. In the covered shoots the amounts of alpha-amino nitrogen in the cortex of the stem were generally greater in the apical than in the basal sections, indicating accumulations in the former tissues resulting possibly from a retardation in the normal processes of new growth due to covering. The amount of alpha-amino nitrogen in the pith of the stems of both the exposed and covered shoots was greater in the apical than in the basal section, being practically twice as great in the apical section of the covered shoots as in the basal section. These results

indicate possibly either an interruption in the movement of this substance to other sections of the plant or non-utilization resulting from retarded growth activity.

4. Rest soluble organic nitrogen, consisting of various unidentified nitrogenous compounds, was present in comparatively great quantities in both exposed and covered shoots. In the B and C groups of leaves of both lots of shoots, rest soluble organic nitrogen increased from the transitional subchlorophyllous (no. 2) to the terminal (nos. 4 and 5) sections. In the basal non-chlorophyllous (no. 1) sections the quantities of rest soluble organic nitrogen were greater than in the transitional (no. 2) sections immediately above. The amounts of this nitrogen fraction were consistently greater in the sections of the leaves of the exposed than in those of the covered shoots.

In the exposed lots of shoots the amounts of rest soluble organic nitrogen were less in the terminal chlorophyllous sections of the more active D group of leaves, possibly because they were utilized more rapidly for new growth in the meristematic tissues of the basal (no. 1) sections, than in comparable sections of such groups of leaves as B and C, which had apparently completed their growth. In the terminal sections of the young E group of leaves of both lots of shoots, the amounts of rest nitrogen were very low, indicating a much higher rate of utilization, and consequently of growth, in the meristematic tissues of the basal sections of this group (E) than in those of the D group of leaves. It will be observed that the value for rest soluble organic nitrogen in the basal non-chlorophyllous (no. 1) section of the young E group of leaves of the covered shoots, composed mostly of meristematic tissues, was considerably higher than for the corresponding section of the exposed shoots, indicating probably that the rate of utilization was not as great in the former as it was in the latter.

The distribution of rest soluble organic nitrogen varied in the cortex and pith of the two sections of the stem of the exposed and covered shoots. The amounts were small in the cortex of the basal section and great in the upper or apical section of the stem of both lots of shoots. The pith of both the apical and basal stem sections of both lots of shoots contained relatively great quantities of rest soluble organic nitrogen.

5. Protein nitrogen accumulated in the various sections of practically all groups of leaves of the exposed shoots in amounts approximately 1.5 to 2.0 times greater than those found in comparable sections of the leaves of the covered shoots. The comparatively greater amounts of chlorophyll in the exposed than in the covered shoots and also the more favorable conditions for photosynthesis and carbohydrate accumulation for the former than for the latter shoots were doubtless factors which also favored the synthesis of proteins. Protein accumulated in greater amounts in the pith of both the apical and basal sections of the stem of the exposed than of the covered

shoots. The difference between the amounts of protein in the cortex of the apical sections of the exposed and covered shoots was very small. In the absence of similar data for the cortex of the basal section of the stem of the covered shoots no contrasts can be made between this portion of the two lots of shoots. If we contrast the distribution of protein in the different sections of the stem of the exposed shoots we find that it is greater in the cortex of the basal than in that of the apical section of the stem, indicating possible storage of this substance in tissues which had completed growth.

### Distribution of sugars

Reducing sugars were determined by Bertrand's method as presented by Kertesz (11). Sucrose was determined after hydrolysis with invertase.

## TABLE IX

Amount of reducing sugars in different sections of the leaves and stem of exposed and covered pineapple sister shoots attached to their mother plant for different periods of time. Based on percentage of fresh weight

| Plant sections | Periods | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 17 days | | 56 days | | 77 days | | 105 days | |
| | Exposed | Covered | Exposed | Covered | Exposed | Covered | Exposed | Covered |
| | % | % | % | % | % | % | % | % |
| Leaves: | | | | | | | | |
| Old: | | | | | | | | |
| B1 (base) | 2.12 | 2.09 | 2.36 | 2.44 | 2.58 | 2.07 | 1.90 | 1.81 |
| B2 | 2.14 | 2.00 | 2.42 | 2.35 | 2.93 | 2.11 | 1.85 | 1.68 |
| B3 | 1.59 | 1.16 | 1.62 | 2.18 | 2.03 | 2.10 | 1.76 | 1.62 |
| B4 (tip) | ..... | ..... | 1.52 | 2.12 | 1.90 | 1.71 | 1.47 | ..... |
| Mature: | | | | | | | | |
| C1 (base) | 1.50 | 2.00 | 2.11 | 2.44 | 2.61 | 2.23 | 2.03 | 1.77 |
| C2 | 1.70 | 1.83 | 1.94 | 2.22 | 2.55 | 2.15 | 1.89 | 1.98 |
| C3 | 1.80 | 1.62 | 1.69 | 1.87 | 2.04 | 1.90 | 1.41 | 1.62 |
| C4 | ..... | ..... | 1.85 | 2.01 | 1.92 | 1.98 | 1.45 | 1.50 |
| C5 (tip) | ..... | ..... | 2.00 | 2.05 | 1.87 | 1.92 | 1.85 | 2.31 |
| Active: | | | | | | | | |
| D1 (base) | ..... | ..... | 1.44 | 2.01 | 1.59 | 2.11 | 1.91 | 1.60 |
| D2 | ..... | ..... | 1.44 | 1.99 | 1.89 | 1.89 | 1.60 | 1.82 |
| D3 | ..... | ..... | 1.13 | 1.73 | 1.96 | 1.73 | 1.81 | 1.49 |
| D4 | ..... | ..... | 1.64 | 1.75 | 2.13 | 1.77 | 1.83 | 1.53 |
| D5 (tip) | ..... | ..... | 2.12 | 1.85 | 2.60 | 2.00 | 1.63 | 1.52 |
| Young: | | | | | | | | |
| E1 (base) | ..... | ..... | 1.73 | 1.44 | 1.64 | 1.28 | 1.80 | 1.08 |
| E2 | ..... | ..... | 1.78 | 1.95 | 1.73 | 1.90 | 1.65 | 2.01 |
| E3 | ..... | ..... | 2.01 | 1.84 | 1.80 | 1.70 | 2.11 | 1.87 |
| E4 + 5 (tip) | ..... | ..... | ..... | 1.95 | 2.52 | 1.54 | 2.17 | 2.01 |
| Stem: | | | | | | | | |
| Basal (cortex) | 1.95 | 1.86 | 2.11 | 1.84 | 1.90 | 1.64 | 1.34 | 1.17 |
| " (pith) | 1.41 | 2.11 | 1.75 | 1.90 | 0.93 | 0.64 | 0.68 | 0.76 |
| Apical (cortex) | ..... | 1.95 | 1.84 | 1.63 | 2.01 | 1.40 | 0.83 | 0.73 |
| " (pith) | ..... | 1.34 | 0.92 | 0.95 | 0.92 | 0.67 | 0.25 | 0.27 |

The distribution of reducing sugars in the various sections of the leaves and stem of exposed and covered shoots removed at the end of all the four different experimental periods is reported in table IX, and that of sucrose in table X. The data on reducing and total sugars and sucrose for the exposed and covered shoots removed at the end of the 56-day period are presented graphically in figure 9.

## TABLE X

AMOUNT OF SUCROSE IN VARIOUS SECTIONS OF THE LEAVES AND STEM OF EXPOSED AND COVERED PINEAPPLE SISTER SHOOTS ATTACHED TO THEIR MOTHER PLANT FOR DIFFERENT PERIODS OF TIME. GIVEN IN PERCENTAGE OF FRESH WEIGHT

| PLANT SECTIONS | PERIODS | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 17 DAYS | | 56 DAYS | | 77 DAYS | | 105 DAYS | |
| | EXPOSED | COVERED | EXPOSED | COVERED | EXPOSED | COVERED | EXPOSED | COVERED |
| Leaves: | % | % | % | % | % | % | % | % |
| Old: | | | | | | | | |
| B1 (base) | 0.43 | 0.35 | 0.33 | 0.29 | 0.35 | 0.26 | 0.24 | 0.25 |
| B2 | 0.35 | 0.35 | 0.34 | 0.38 | 0.55 | 0.33 | 0.35 | 0.22 |
| B3 | 0.83 | 0.51 | 0.59 | 0.52 | 0.63 | 0.64 | 0.74 | 0.33 |
| B4 (tip) | ..... | ..... | 0.77 | 0.65 | 0.93 | 0.70 | 1.08 | ..... |
| Mature: | | | | | | | | |
| C1 (base) | 0.35 | 0.35 | 0.27 | 0.30 | 0.40 | 0.29 | 0.32 | 0.28 |
| C2 | 0.20 | 0.28 | 0.25 | 0.60 | 0.31 | 0.29 | 0.32 | 0.23 |
| C3 | 0.80 | 0.32 | 0.60 | 0.54 | 0.72 | 0.34 | 0.83 | 0.21 |
| C4 | ..... | ..... | 0.98 | 0.19 | 0.95 | 0.35 | 0.85 | 0.22 |
| C5 (tip) | ..... | ..... | 1.22 | 0.59 | 1.03 | 0.58 | 1.19 | 0.65 |
| Active: | | | | | | | | |
| D1 (base) | ..... | ..... | 0.48 | 0.40 | 0.24 | 0.35 | 0.44 | 0.23 |
| D2 | ..... | ..... | 0.24 | 0.34 | 0.21 | 0.21 | 0.38 | 0.21 |
| D3 | ..... | ..... | 0.25 | 0.14 | 0.41 | 0.15 | 0.74 | 0.14 |
| D4 | ..... | ..... | 0.49 | 0.14 | 0.78 | 0.18 | 1.18 | 0.19 |
| D5 (tip) | ..... | ..... | 0.97 | 0.23 | 1.02 | 0.00 | 1.28 | 0.18 |
| Young: | | | | | | | | |
| E1 (base) | ..... | ..... | 0.46 | 0.30 | 0.40 | 0.25 | 0.43 | 0.25 |
| E2 | ..... | ..... | 0.27 | 0.25 | 0.26 | 0.24 | 0.19 | 0.22 |
| E3 | ..... | ..... | 0.85 | 0.22 | 0.19 | 0.19 | 0.68 | 0.16 |
| E4 + 5 (tip) | ..... | ..... | ..... | 0.15 | 0.64 | 0.20 | 0.97 | 0.19 |
| Stem: | | | | | | | | |
| Basal (cortex) | 0.61 | 0.49 | 0.42 | 0.44 | 0.52 | 0.53 | 0.32 | 0.18 |
| " (pith) | 0.72 | 0.86 | 0.63 | 0.68 | 0.54 | 0.58 | 0.31 | 0.20 |
| Apical (cortex) | ..... | 0.66 | 0.76 | 0.62 | 0.71 | 0.50 | 1.41 | 0.33 |
| " (pith) | ..... | 0.93 | 0.70 | 0.74 | 0.83 | 0.68 | 1.16 | 0.46 |

Examination of the data in table IX shows, with certain exceptions, that the amounts of reducing sugars in the sections of the leaves of the exposed shoots were not appreciably greater than those of the covered shoots. The amounts of reducing sugars in the cortex of both the apical and basal sec-

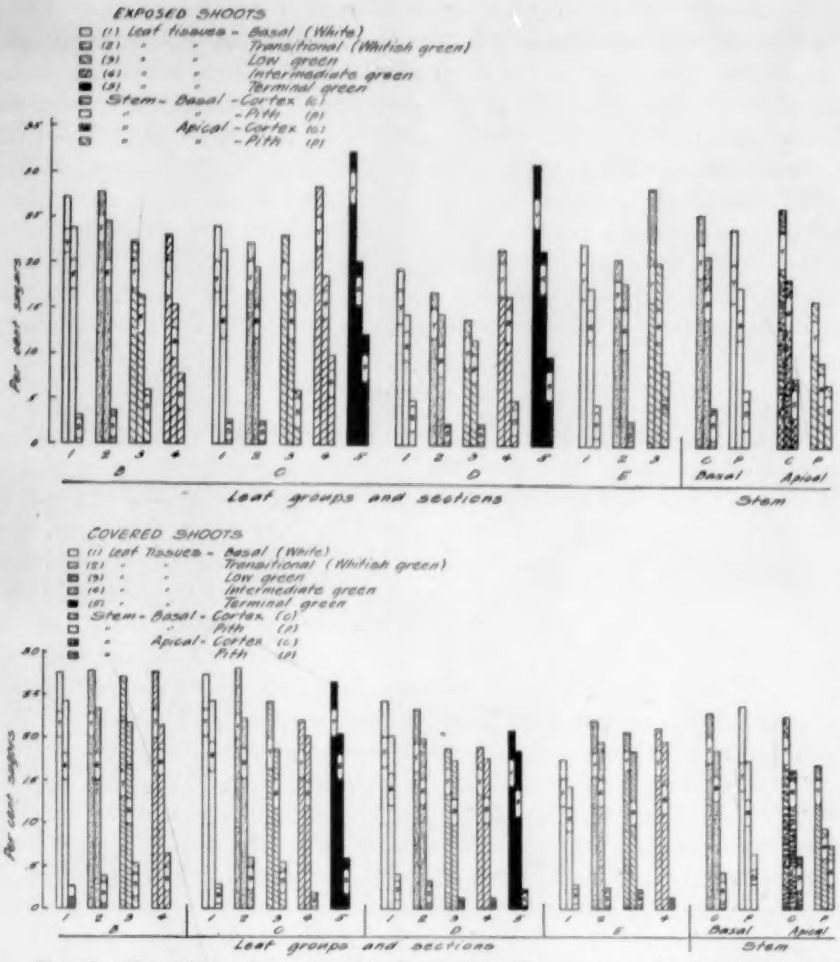

EXPOSED SHOOTS
(1) Leaf tissues - Basal (White)
(2) " " Transitional (Whitish green)
(3) " " Low green
(4) " " Intermediate green
(5) " " Terminal green
Stem - Basal - Cortex (c)
" " Pith (p)
" Apical - Cortex (c)
" " Pith (p)
Per cent sugars
Leaf groups and sections
Stem
COVERED SHOOTS
(1) Leaf tissues - Basal (White)
(2) " " Transitional (Whitish green)
(3) " " Low green
(4) " " Intermediate green
(5) " " Terminal green
Stem - Basal - Cortex (c)
" " Pith (p)
" Apical - Cortex (c)
" " Pith (p)
Per cent sugars
Leaf groups and sections
Stem

FIG. 9. Percentage of sucrose, reducing, and total sugars on a fresh weight basis in different sections of the leaves and stem of exposed (A) and covered (B) pineapple shoots at the end of the 56-day period.

tions of the stem were greater for the exposed than for the covered shoots. The distribution of the quantities of these sugars in the pith was very irregular. This may be explained on the supposition that reducing sugars are readily converted in the pith—a storage organ—into either sucrose or starch, the two latter being more suitable as reserve substances than the former.

The data on the distribution of sucrose in comparable sections of the leaves and stem of the two lots of shoots show, according to the values in

table X, greater amounts for the exposed than for the covered shoots. There are a few exceptions, occurring mostly in the basal non-chlorophyllous sections of the leaves and in the basal section of the stem, which cannot be explained satisfactorily. The greater amounts of sucrose in the chlorophyllous sections (nos. 3, 4, and 5) of practically all the groups of leaves of the exposed shoots constitute the only positive evidence of sugar accumulations under conditions favorable for photosynthesis. Also, the greater plant weights and greater accumulations of proteins in the sections of the leaves of the exposed than in the sections of the leaves of the covered shoots, produced at the expense of leaf sugars or of other carbohydrates, constitute additional evidence of greater carbohydrate synthesis in the former than in the latter shoots.

### Calcium

Calcium was determined for purposes of comparison with nitrate, since the bulk of the nitrogenous salts were supplied to the experimental mother plants as $Ca(NO_3)_2$. Analytical data for the shoots removed at the end of the 105-day period are presented in table XI. They show, in general, that calcium was absorbed in considerably greater quantities by the exposed than by the covered shoots. These quantities were from 1.18 to 12.33 times greater in the various sections of the leaves of the exposed than of the covered shoots. The stem contained greater amounts of calcium than the leaves. Most of the calcium in the stem was confined to the pith, which contained approximately from 1.4 to 2.0 times more calcium than the cortex. The amounts of calcium in the stem sections of the exposed shoots were from 1.19 to 1.45 times greater than in the covered shoots. The data indicate generally that the movement of calcium from the mother plant to the stem of the covered shoots was not as much interrupted as its translocation from pith to cortex and then to leaves. The quantities of calcium in the leaves increased from the transitional (no. 2) to the terminal (no. 5) sections, being in many cases slightly higher in the basal non-chlorophyllous (no. 1) than in the transitional (no. 2) sections. The sections of leaves of chronologically and physiologically advanced groups, excepting the old and senile B group of the exposed shoots, contained, in general, more calcium than those of the less mature and young leaf groups. The distribution of calcium in the different leaf groups of the covered shoots did not vary as much as it did in those of the exposed shoots.

## Discussion

The experimental data presented indicate that the growth and physiological behavior of two sister shoots, one exposed to light and the other kept in darkness while attached to their mother plant, were influenced considerably by these conditions.

## TABLE XI

MILLIGRAMS OF CALCIUM PER GRAM OF FRESH WEIGHT IN VARIOUS SECTIONS OF THE LEAVES AND STEM OF EXPOSED AND COVERED PINEAPPLE SHOOTS ATTACHED TO THEIR MOTHER PLANT FOR 105 DAYS

| PLANT SECTIONS | EXPOSED SHOOTS | COVERED SHOOTS | RATIO: EXPOSED / COVERED |
|---|---|---|---|
| | *mg.* | *mg.* | |
| Leaves: | | | |
| Old: | | | |
| B1 (base) | 2.000 | 1.700 | 1.18 |
| B2 | 2.200 | 0.900 | 2.45 |
| B3 | 2.900 | 0.600 | 4.82 |
| B4 (tip) | 5.200 | ..... | ..... |
| Mature: | | | |
| C1 (base) | 2.700 | 0.600 | 4.50 |
| C2 | 2.000 | 0.400 | 5.00 |
| C3 | 3.700 | 0.300 | 12.33 |
| C4 | 4.800 | 0.400 | 12.00 |
| C5 (tip) | 7.800 | 0.800 | 9.75 |
| Active: | | | |
| D1 (base) | 1.900 | 0.400 | 4.75 |
| D2 | 1.500 | 0.500 | 3.00 |
| D3 | 1.700 | 0.500 | 3.40 |
| D4 | 2.900 | 0.800 | 3.63 |
| D5 (tip) | 5.400 | 1.000 | 5.40 |
| Young: | | | |
| E1 (base) | 1.000 | 0.500 | 2.00 |
| E2 | 1.200 | 0.600 | 2.00 |
| E3+4 | 1.600 | 0.600 | 2.67 |
| E4+5 (tip) | 3.400 | 0.800 | 4.25 |
| Stem: | | | |
| Basal (cortex) | 5.800 | 4.000 | 1.45 |
| " (pith) | 9.500 | 8.200 | 1.19 |
| Apical (cortex) | 7.400 | 5.600 | 1.32 |
| " (pith) | 11.340 | 8.000 | 1.42 |

Judging from the behavior of the exposed and covered shoots, one may safely state that the chlorophyllous leaf tissues of the covered shoots suffered from serious physiological injury because formation of new chlorophyll was inhibited and that already present underwent some decomposition. With the onset of this latter condition, especially in the terminal sections of aged leaves, a gradual dehydration and death of the tissues involved followed. Any movement of organic substances from the mother plant to the shoots possibly aided only the growth and maintenance of the tissues of the non-chlorophyllous sections of the leaves and the meristematic ones of the stem, but not the highly differentiated chlorophyllous tissues of the leaves.

The shoots kept in darkness were more succulent, that is, they contained greater amounts of water per unit of fresh tissues, because they were unable to increase total organic matter as well as the solute content of their sap with the cessation of photosynthesis.

The data on the distribution of calcium, nitrate, titratable acidity and soluble organic nitrogen are in harmony with those of electrical resistance. The values of electrical resistance for the sections of the covered shoots were, with a few exceptions, higher than those for comparable sections of the exposed shoots, indicating smaller amounts of electrical-conducting solutes in the sap of the former shoots.

The chlorophyllous sections (nos. 3, 4, and 5) of the leaves of exposed shoots contained approximately from 2 to 10 times greater amounts of titratable acidity than comparable sections of the leaves of the covered shoots. The extremely small amounts of titratable acidity in covered shoots may be attributed to cessation of organic acid synthesis in the absence of light and to the utilization of the previously produced and reserve organic acids in the tissues by the protoplasm either as a source for energy or for other metabolic processes. The data suggest that there are possibly two distinct types of physiological processes for the generation of organic acids in the non-chlorophyllous and in the chlorophyllous tissues of pineapple leaves and of the stem. It appears that the processes involved in the generation of organic acids in the chlorophyllous tissues of the leaves are closely associated with those of photosynthesis because they are favored in the presence and are inhibited in the absence of light or possibly of carbohydrates. Whereas, the processes which favor the formation of organic acids in the stem and in the non-chlorophyllous tissues of the leaves appear to be of a respiratory nature, possibly resulting from the incomplete oxidation of carbohydrates. SPOEHR (**25**) has reviewed the literature on organic acid synthesis and mentions briefly in his book (chapter 5) the theory of Liebig, who considered organic acids as intermediate products in the synthesis of carbohydrates, and that of ROCHLEDER, who assumed that carbon dioxide was reduced to carbon monoxide and the latter was converted to formaldehyde and then to formic acid, which, uniting with carbon dioxide, formed oxalic acid. RUHLAND and WOLF (**20**) have concluded their review of this subject as follows "... malic acid metabolism is probably linked with that of carbohydrates, either in the process of respiration or of photosynthesis." BENNET-CLARK (**2, 3**) has proposed, in his extensive review and critique of the literature on the rôle of organic acids in the metabolism of plants, a reconsideration of Baeyer's hypothesis, in favor of that of Liebig, for a more rational interpretation of the mechanism of photosynthesis in view of recent studies (**2**) which have suggested that malic acid is converted to carbohydrates and that different concentrations found at different times of day are due, in part, to temperature differences and possibly also to different oxygen tensions in the tissues. VICKERY *et al.* (**33**) have followed the fate of malic acid in excised tobacco leaves kept in nutrient solutions and in darkness for 159 to 279 hours, and state that the amounts of malic acid which disappeared

were greater than those of asparagine found in the tissue, and also that the total acidity did not change significantly. They also observed that the amounts of citric acid increased almost in direct proportion with the amounts of malic acid which had disappeared, this suggesting transformation of malic to citric acid. Our findings suggest that an entirely different kind of mechanism for the disappearance of organic acids operated in pineapple leaves than that observed by VICKERY *et al.* (**33**) in tobacco leaves because the quantities of acids in pineapple leaves decreased in practically all cases.

The data suggest, in general, that the organic acid reserves in the chlorophyllous sections of the leaves may act, possibly after decarboxylation, as sources of energy in the metabolism of pineapple plants.

The amounts of nitrate in the stem and in the non-chlorophyllous tissues were comparatively very high, indicating that assimilation of nitrate in these sections was either very slight or probably non-existent. The etiolated chlorophyllous sections of the leaves of the covered shoots contained comparatively large amounts of nitrate, indicating that the rate of nitrate assimilation had decreased considerably in the absence of chlorophyll and light and possibly because of a lack of sufficient amounts of carbohydrates. The influence of light and of chlorophyll and carbohydrate content on the assimilation of nitrate in the chlorophyllous sections of leaves cannot be segregated in physiological investigations in determining the specific agent or agents for the activation of the mechanism of nitrate assimilation. ANDERSON (**1**) observed that the amounts of nitrate in the etiolated leaves were higher than those found in the green leaves of wheat, oats, and cabbage. These findings are in harmony with ours. SCHIMPER, according to MILLER (**13**) found that nitrates became depleted in green leaves exposed to daylight but that they did not do so in leaves kept in the dark; also that nitrates disappeared in green but not in etiolated leaves. WEBSTER (**34**) found that the roots and stem of soy-bean plants contained, at about the time the seeds matured, greater amounts of nitrate than the leaves. The literature, as reviewed in the first paper of this series (**21**), shows that nitrate is reduced in different plants in light as well as in darkness, and in the presence as well as in the absence of chlorophyll. These generalizations, however, may not hold for all organs of all plants. Indeed, our evidence from the pineapple plant suggests that in the roots, stem, and non-chlorophyllous sections of leaves of pineapple plants nitrate is assimilated either very slowly or probably not at all, while in the chlorophyllous sections of the leaves (with the exception of etiolated leaves kept in the dark) nitrate assimilation is very rapid. The set of conditions essential for the assimilation of nitrate by pineapple plants may differ considerably from that for other plants belonging to different orders and families. If this point of view, *i.e.*, that the set of conditions necessary for the assimilation of nitrate is different for different plants, is

taken into consideration, it may help us in finding order in the chaos of contradictory evidence.

The distribution of reducing sugars in the various sections of the leaves and stem of the exposed and covered shoots show, as may be seen in table IX, that reducing sugars were present in greater quantities in the cortex of the exposed than in that of the covered shoots, while the values for all other sections were inconsistent. With respect to sucrose distribution, the values in most plant sections were higher for the exposed than for the covered plants. These results are in agreement with those of STANESCU (**26**) who observed that in the leaves of *Acer negundo* and *Medicago sativa* the amounts of monosaccharides remained practically unchanged during both day and night, while the amounts of disaccharides diminished appreciably at night and showed a marked increase during the day. Variations in the sugar content of different plant sections possibly indicate either a high or a low rate of anabolic or catabolic processes, or some specialization in the physiological function of the section or organ. The variation in the content of sucrose or of other substances in different sections may be interpreted if one takes into consideration the photosynthetic ability of different leaf groups, the physiological functions of different sections and the growth activities of the plant at its different stages of development. For instance, sucrose was found in greater quantities in the terminal sections (nos. 4 and 5) of the mature C and fully expanded D group of leaves of the exposed shoots than in the E group of leaves, because of rapid sucrose utilization in the rapidly growing basal tissues of the latter group of leaves. The greater amounts of sucrose in the C and D groups of leaves and smaller amounts in the B group may be attributed to greater protoplasmic vigor and photosynthetic activity in the former than in the latter leaves. PARKIN (**16**) observed in *Galanthus nivalis* that the sugar content of the leaf increased from the terminal to the basal sections, while the ratio of sucrose to hexose diminished in the same direction. This corresponds approximately with the results obtained for pineapple leaves.

The distribution of calcium, as presented in table XI, shows that this substance was absorbed in greater amounts by the exposed than by the covered shoots. The amounts of calcium and of nitrate in the non-chlorophyllous sections of the leaves and stem of both exposed and covered shoots ran parallel in all groups of leaves, suggesting that both substances were absorbed at approximately equal rates. In the chlorophyllous sections of the leaves no comparison can be made between calcium and nitrate content, owing to the disappearance of the latter through assimilation. No claim is made that calcium and nitrate were absorbed in equimolecular proportions, because other unpublished data have clearly demonstrated that nitrate, under certain conditions, is absorbed at a greater rate than calcium. The

fact that calcium accumulates in the apical section of the stem is of great interest. Because of probable incomplete development of conducting vessels in this meristematic tissue, as is true in other plants, movement of solutes is by diffusion only and is, therefore, slow so that accumulation results. STEWART and ARTHUR (**28**) have observed that decreased light intensity during the summer increased the dry weight, ash, and phosphorus, but decreased the calcium content in *Lycopersicum esculentum*. HIBBARD and GRIGSBY (**9**) also found that potassium and calcium accumulated more rapidly in light than in darkness. FREELAND (**8**) observed that plants with a high transpiration rate, to which category our exposed shoots would belong, had higher values for dry weight, ash, calcium, phosphorus and potassium contents than plants with a low transpiration rate.

It is also of interest to note that the amounts of organic acids in the leaf tissues, reported as titratable acidity, correlated with those of calcium, as found by CLARK (**6**) for the tomato plant and VICKERY *et al.* (**33**) for the tobacco plant.

## Summary

Forty pairs of sister shoots, one pair per mother plant, were selected in the period from July 20 to 23, 1934, for uniformity and vigor after the ripe fruits of the mother plants had been removed. One of the shoots from each pair was covered with a bag made of green oil cloth which fitted very loosely around the shoot; the other was left exposed. Both covered and exposed sister shoots were left attached to their mother plants for periods of 17, 56, 77, and 105 days, respectively. Between 5 and 10 pairs of sister shoots, representing equal numbers of covered and exposed shoots, were harvested at the end of each period and were studied for growth, distribution of moisture, chlorophyll, electrical resistance, acidity, calcium, and nitrogen and sugar fractions in different sections of the leaves and stem. The results were as follows:

1. The weights of the covered shoots were less at the end of the 77- and 105-day periods than those of the exposed shoots, indicating retardation but not necessarily complete cessation of growth.

2. The moisture content of the various sections of the leaves and stem was consistently greater for the covered than for the exposed shoots, indicating that the total solids, including organic and inorganic substances, per unit of fresh tissues, were greater for the exposed than for the covered shoots.

3. There was a breaking down of chlorophyll in the leaves of covered shoots which had developed before covering and chlorophyll did not form in the leaves that were produced after covering. The chlorophyll content of the exposed shoots varied from one period to another.

4. The electrical resistance of the extracted sap, reported in ohms, was greater for the covered than for the exposed shoots, indicating a greater

content of electrolyte solutes in the sap for the exposed than for the covered shoots.

5. Titratable acidity, reported as percentage of citric acid, was greater in the chlorophyllous sections of the exposed than of the covered shoots. In the non-chlorophyllous sections of the leaves and in the stem, these values were higher for the covered than for the exposed shoots. The results indicate that the titratable acidity content of the chlorophyllous sections of the covered shoots did not increase during the period in which they were covered, but decreased instead, probably as a result of an inadequate carbohydrate supply. The smaller amounts of organic acids in the chlorophyllous sections of the covered shoots were attributed to decarboxylation and the utilization of the by-products by the protoplasm, either as a source of energy or for the synthesis of more complex compounds. The production of organic acids in the chlorophyllous sections of the leaves appeared to be associated either directly or indirectly with photosynthetic processes, since it was favored by light and inhibited by darkness. The generation of organic acids in the non-chlorophyllous tissues of the stem and leaves is of a respiratory type, because organic acid formation was favored by darkness.

6. Nitrate nitrogen was more abundant in the non-chlorophyllous sections of the leaves and in the stem of the exposed than in those of the covered shoots, indicating a higher rate of absorption in the former than in the latter shoots. Values for total organic nitrogen were generally higher for the exposed than for the covered shoots, which is in harmony with the data on nitrate absorption. The relative rate of nitrate assimilation decreased considerably in the chlorophyllous sections of the leaves of the covered shoots, as indicated by the amounts of unassimilated nitrate; this may also account for the lower values of organic nitrogen in the covered than in the exposed shoots.

7. There was no outstanding difference in the amounts of reducing sugars found in the exposed and in the covered shoots, which indicates that these substances were either translocated to other parts of the plant or were constantly regenerated from other more complex compounds stored in the tissues. Sucrose was consistently present in greater amounts in the tissues of the exposed than in those of the covered shoots. Variations in the sucrose content of different leaf and stem sections are discussed.

8. Calcium was found in greater quantities in the leaf and stem sections of exposed than of covered shoots, indicating that absorption was greater by the former than by the latter shoots. The amounts of calcium were greater in the pith of the apical section of the stem than in any other section. It is suggested that these large amounts of calcium in the meristematic tissues of the stem may be the result of accumulation due to incomplete development of conducting vessels.

9. The data show, in general, that sister shoots receiving a great amount of their inorganic nutrition and water supply by way of the roots and stem of the mother plant differ considerably in their physiological behavior and chemical composition when one is exposed to light and the other is kept in darkness.

## LITERATURE CITED


1. Anderson, V. L. Some observations on the nitrate reducing properties of plants. Ann. Bot. **38**: 699–706. 1924.
2. Bennet-Clark, T. A. The rôle of the organic acids in plant metabolism. New Phytol. **32**: 37–71; 128–161; 197–230. 1933.
3. ————————. Organic acids in plants. Ann. Rev. Biochem. **6**: 579–594. 1937.
4. Brown, W. H., and Trelease, S. F. Alternate shrinkage and elongation of growing stems of *Cestrum nocturnum*. Philippine Jour. Sci. C. **13**: 353–360. 1918.
5. Burkholder, P. R. The rôle of light in the life of plants. Bot. Rev. **2**: 1–52; 97–172. 1936.
6. Clark, H. E. Effect of ammonium and of nitrate nitrogen on the composition of the tomato plant. Plant Physiol. **11**: 5–24. 1936.
7. Eisenmenger, W. S. The distribution of nitrogen in tobacco when the supplies of nitrogen and of light are varied during the growing period. Jour. Agr. Res. **46**: 255–265. 1933.
8. Freeland, R. O. Effect of transpiration upon the absorption and distribution of mineral salts in plants. Doctor's Dissertation, no. 17. The Ohio State University Press. 1935.
9. Hibbard, R. P., and Grigsby, B. H. Relation of light, potassium, and and calcium deficiencies to photosynthesis, protein synthesis translocation. Michigan Agr. Exp. Sta. Tech. Bull. 141. 1934.
10. Ivanoff, M. Versuche über die Frage, ob in den Pflanzen bei Lichtabschluss Eiweisstoff sich bilden. Land. Vers.-Sta. **55**: 78–94. 1901.
11. Kertesz, Z. I. Recalculated tables for the determination of reducing sugars by Bertrand's method. B. Westermann Co., New York. 1930.
12. Kishi, Y., and Yokota, Y. Studies in the change of chemical constituents of mulberry leaves in the intercepted sunlight. (English Sum.) Bull. Sci. Fak. Terkult Kyusu Imper. Univ. **6**: 103–104. 1935.
13. Miller, E. C. Plant physiology. McGraw-Hill Book Co., New York. 1931.

14. NIGHTINGALE, G. T., and SCHERMERHORN, L. G. Nitrate utilization by asparagus in the absence of light. Science n.s. **64**: 282. 1926.

15. ————, ————, and ROBBINS, W. R. The growth status of the tomato as correlated with organic nitrogen and carbohydrates in roots, stems and leaves. New Jersey Agr. Exp. Sta. Bull. **461**: 1–38. 1928.

16. PARKIN, J. The carbohydrates of the foliage leaf of the snow drop (*Galanthus nivalis*) and their bearing on the first sugar of photosynthesis. Biochem. Jour. **6**: 1–47. 1912.

17. PRIESTLEY, J. H. Light and growth. III. An interpretation of phototropic growth curvatures. New Phytol. **25**: 213–247. 1926.

18. ————, and EWING, J. Physiological studies in plant anatomy. VI. Etiolation. New Phytol. **22**: 30–44. 1923.

19. RICHARDS, H. M. Acidity and gas interchange in cacti. Carnegie Inst. Washington, Pub. no. 209. 1915.

20. RUHLAND, WILHELM, and WOLF, J. Metabolism of carbohydrates and organic acids in plants. Ann. Rev. Biochem. **5**: 485–512. 1936.

21. SIDERIS, C. P., KRAUSS, B. H., and YOUNG, H. Y. Assimilation of ammonium and nitrate nitrogen from solution cultures by roots of *Pandanus veitchii* Hort. and distribution of the various nitrogen fractions and sugars in the stele and cortex. Plant Physiol. **12**: 899–928. 1937.

22. ————. Assimilation of ammonium and nitrate by pineapple plants grown in nutrient solutions and its effects on nitrogenous and carbohydrate constituents. Plant Physiol. **13**: 489–527. 1938.

23. ————. Distribution of different nitrogen fractions, sugars and other substances in various sections of the pineapple plant grown in soil cultures and receiving either ammonium or nitrate salts. Plant Physiol. **14**: 227–254. 1939.

24. ————. Distribution of nitrogen fractions, sugars, and other substances in *Ananas*, grown in darkness versus daylight. Plant Physiol. **14**: 647–676. 1939.

25. SPOEHR, H. A. Photosynthesis. Chemical Catalogue Co., New York. 1926.

26. STANESCU, P. P. Les variations quantitatives des produits de la photosynthése dans les feuilles des plantes vertes pendant un jour (24 heures). Thèse, Université de Bucarest, 1927.

27. STREET, O. E. Carbohydrate-nitrogen and base element relationships of peas grown in water culture under various light exposures. Plant Physiol. **9**: 301–322. 1934.

28. STEWART, W. D., and ARTHUR, JOHN M. Change in mineral composition of the tomato plant irradiated with a quartz-mercury vapor

lamp and its relation to the level and ratio of calcium and phosphorus in the nutritive medium. Contrib. Boyce Thompson Inst. **9**: 105–120. 1937.

29. TOLMACHEV, IVAN. Effect of darkness and light on the organic acids in the plant. Lapiski Kiiv. Sil's Ko-Gospod. Inst. (Kyiv. Agr. Inst. Mem.) **2**: 1–21. 1927.
30. TOTTINGHAM, W. E., and LEASE, E. J. A photochemical aspect of nitrate assimilation in plants. Science n.s. **80**: 615–616. 1934.
31. TRELEASE, S. F. Night and day rates of elongation of banana leaves. Philippine Jour. Sci. **23**: 85–96. 1923.
32. VICKERY, H. B., PUCHER, G. W., LEAVENWORTH, C. S., and WAKEMAN, A. J. Chemical investigations of the tobacco plant. V. Chemical changes that occur during growth. Connecticut Agr. Exp. Sta. Bull. **374**: 553–619. 1935.
33. ———, ———, ———, and ———. Chemical investigations of the tobacco plant. VI. Chemical changes that occur in leaves during culture in light and darkness. Connecticut Agr. Exp. Sta. Bull. **399**: 753–832. 1937.
34. WEBSTER, J. E. Nitrogen metabolism in the soy bean. Plant Physiol. **3**: 31–43. 1928.
35. WOLF, JOHANNES. Beitrag zur Kenntnis des Saürestoffwechsels succulenter Crassulacaen. Planta **15**: 572–644. 1931.

# RESPIRATION OF CEREAL GRAINS AND FLAXSEED[1]

C. H. BAILEY

(WITH FIVE FIGURES)

## Introduction

In earlier papers by BAILEY (**2, 3**) and BAILEY and GURJAR (**4, 5a, 5b, 5c, 5d**), the researches in this field prior to 1921 were reviewed, and the relationship of moisture content, damaged and broken kernels, and various other factors to the relative level of respiration of wheat, rice, and corn (maize) was traced. These laboratory investigations paralleled or supplemented certain large scale, or practical, studies of grain storage. It became evident that the prediction of the behavior of stored grain as determined by chemical composition, physical characteristics, and the several biological factors involved in keeping qualities, is not a simple matter. The weighting of these several variables, and the extent to which they are related to factors of environment, including climatic conditions, composition of the air in the grain bin, and ventilation becomes very involved. Despite these practical difficulties, it still appears that the laboratory attack upon certain of the details of this problem is a logical procedure and will ultimately lead to the most satisfactory solutions.

Observations made during commercial operations of grain handling and storage, while interesting and useful as respects the individual parcels of grain involved, are of limited value in effecting an analysis of the several factors operative in such instances. Thus there is a definite lack of control of many variables in these cases, certain of which cannot even be held constant during any extended period. Moreover, there is also the formidable item of expense when the storage of thousands of bushels of grain is involved, which places such work outside of the reach of modest research budgets.

Since the publication of the papers cited above, several contributions to the literature have appeared, and certain of these tie into the researches reported in this paper so definitely as to necessitate a brief review of them before detailing the data accumulated by the author. COLEMAN, ROTHGEB, and FELLOWS (**10**) observed much the same relationships between moisture content, and other factors of quality of sorghum, and the rate or respiration as measured in terms of $CO_2$ produced per unit of time and material, that had previously been reported for corn (maize) by BAILEY (**2**). The actual levels of respiration were lower in sorghum than in maize, however.

BAKKE and NOECKER (**6**) studied the relation of moisture to respiration

[1] These studies were made possible by a grant from the Research Funds of the Graduate School of the University of Minnesota.

and heating in stored oats. They concluded that heating is negligible when the moisture is below 15.0 per cent. The same rapid increase in rate of respiration as the moisture increased was observed in this study as had previously been found to be true of corn, wheat, and sorghums. The high temperatures developed in moist grain during self-heating was found to lower the vitality of the seed. These authors attributed temperature variations of various samples at the same moisture level to variations in the fungal flora of different seed lots.

BROWNE (**7**) gives a good historical review of the early observations of the spontaneous heating of agricultural products and a critical discussion of past and present theories concerning the phenomenon. He points out that although heating is a common occurrence, the exact coincidence of all the conditions necessary for the spontaneous ignition of agricultural products is fortunately infrequent. He indicates that more information is needed on these subjects, such as the elementary analysis of the heating material, analysis of the solid, liquid and gaseous organic compounds that are produced in both anaerobic and aerobic fermentation, and knowledge of moisture migration and heat transference.

AGRONOMOFF (**1**) engaged in an extensive study of sound, dry cereal grains stored in large elevator bins. He observed that the thermal conduction of such material is low. The elevation of temperature in the summer is accordingly a function of the thickness of the layer where the grain is not disturbed. In the instance of moist oats containing 14.8 per cent. water, there was a substantial increase in temperature during the summer as contrasted with dry oats containing 11.0 per cent. water. Moist grain during the summer should not be placed in outside or corner silos, he concluded. He also comments on the increased development of granary weevils in consequence of the elevation of temperature in spring and summer.

GILMAN and BARRON (**12**) conclude that the temperatures reached in a bin of stored grain are due to the interaction of several factors. These include the "heat of germination" itself and temperatures generated by the growth of molds developing on the grain saprophytically. JAMES, RETTGER, and THOM (**16**) as part of their study of microbial thermogenesis had previously discussed the production of heat which accompanies the growth of micro-organisms, contending that in the presence of air or oxygen micro-organisms are capable of producing comparatively high temperatures. The fact that molds may effect an elevation of temperature when grown on suitable organic substances had been shown repeatedly prior to this study.

GILMAN and BARRON (**12**) undertook their investigation with the object of isolating and evaluating the rôle of each of these two sources of heat in the temperature increase occurring in stored grains. They compare the temperatures reached when sterilized oats, wheat, and barley at 18 to 30 per

cent. moisture levels are stored in Dewar flasks. The seeds were inoculated with three species of Aspergillus while uninoculated seeds served as controls. In these experiments the temperature of the check samples remained approximately the same during a six-day period while the samples with fungal growth rose rapidly from a temperature of 30° to 50° C. In this connection WILSON (**22**) has suggested that a temperature of 30° C. affects the protoplast of the kernel in a manner which permits the outward leaching of soluble food materials that are readily attacked by the fungi commonly present.

SWANSON (**21**) states that a relatively high moisture content will accelerate respiration in the grain and favors the growth of molds, both of which will generate heat. The percentage of moisture at which molds developed was closely related to the temperature, since there was no mold growth on wheat stored at 60° F. even though the moisture content of the wheat was 18 per cent. Mold growth is considered an indication of damage in stored grain.

ROZSA (**19**) discussed PAPS's investigation which indicated that moldiness did not become a prominent factor in wheat spoilage until the relative humidity of atmospheres in hygroscopic equilibrium with the grain exceeded 75 per cent. This level of humidity is in equilibrium with wheat containing about 15 per cent. of moisture according to the average of PAPS's data, which does not agree with that of COLEMAN and FELLOWS (9), since the latter found that 17 to 17.5 per cent. of moisture was in equilibrium with a 75 per cent. relative humidity. Moreover, SKOVHOLT and BAILEY (**20**) found that common molds, including *Penicillium expanium* and *Rhizopus nigricans,* were very sensitive to humidity levels and failed to grow on bread crust until the humidity was in excess of 85 per cent. and the moisture content of the material about 20 per cent. *Aspergillus niger* grew at a slightly lower level of humidity but only to a very slight degree at 85 per cent. humidity. Incidentally, a humidity of 85 per cent. was found by COLEMAN and FELLOWS (9) to be in equilibrium with wheat having a moisture content of about 21.5 per cent. although PAPS's findings placed the equilibrium point at about 18 per cent. moisture.

Even though these several studies do not entirely agree, it does appear that an acceleration of respiration of bulk wheat in consequence of mold growth is not likely to occur until the moisture content of the grain exceeds that which is safe for ordinary storage. In the writer's judgment molds will not proliferate freely until the moisture content of wheat is in excess of 17.5 per cent. moisture, which is substantially above the level permitted under the U. S. Grain Standards for the grades commonly accepted for delivery under contract.

LARMORE, CLAYTON, and WRENSHALL (**14**) studied the respiration of

hard spring wheat. They realized that the true respiration of wheat is complicated by the respiration of fungi which develops on damp wheat. In this study the germination and growth of fungi was controlled effectively by toluene and carbon tetrachloride vapors. In the presence of these substances carbon dioxide was produced at a low rate and no heating occurred in wheat of 25 per cent. moisture content.

## Methods

Samples of grain to be tested were brought to approximately 11, 13, 15, and 17 per cent. moisture levels by the addition of water to weighed portions of dry (11 per cent. moisture or less) grain contained in large, tightly-stoppered bottles. While moisture equilibrium was being attained the samples were stored at 2° C. to restrain the growth of micro-organisms until after the surface water had been absorbed by the interior tissues of the kernels. After three or four days of such storage the samples were brought to room temperature and thoroughly mixed, by using a grain divider, and the desired quantity (from 250 to 500 grams, depending upon the moisture content) of the grain was weighed into the respiration chambers.

The moisture content of the sample was determined, at the same time, by weighing out into Petri dishes duplicate 20-gram portions of grain. These samples were allowed to air dry in the laboratory and the loss in weight was noted carefully. The air-dried samples were then ground in a burr mill and two-gram portions weighed up for the final determination of moisture by the Association of Official Agricultural Chemists (1937) air oven method. This double moisture determination, while time consuming, is necessary if reliable moisture determinations are to be obtained on wetted grain.

Several duplicate grain samples were placed in sheet-metal towers fitted with an inlet and outlet tube and otherwise tightly sealed. All rubber connections were thoroughly paraffined since carbon dioxide may diffuse through rubber not protected in some such manner. The metal grain cylinders were placed in an air thermostat maintained at 37.8° C. (100° F.) which is the temperature at which the earlier investigations were conducted by Bailey *et al.*

The essential features of the apparatus for the absorption of carbon dioxide respired by grain are shown in figure 1. Entering air was passed through a gas meter in order to determine the total volume of gas displaced in the system. In all determinations sufficient air was admitted to replace four times the total volume of gas in the entire system. The incoming air was freed from carbon dioxide by conducting it through a large soda lime tower. This $CO_2$-free air was then passed through a series of bottles containing sulphuric acid of the proper specific gravity to bring the escaping air to a relative humidity in equilibrium with the hygroscopic moisture content of the grain to be aspirated in order to avoid moisture changes in the

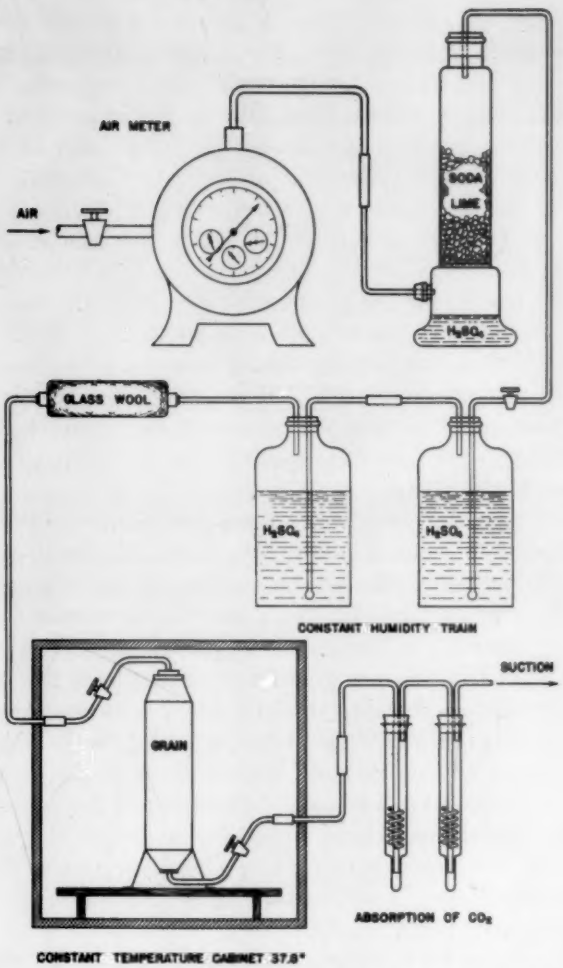



FIG. 1. Diagram of apparatus used in determining the rate of respiration of grain.

grain during the aspiration process. The data for adjusting atmospheres to the appropriate relative humidity were taken from the papers by COLEMAN and FELLOWS (9), and WILSON (22).

The humidified $CO_2$-free air forced the accumulated $CO_2$ from the respiration chamber into a measured quantity of standard barium hydroxide solution contained in specially constructed absorption vessels as indicated in the accompanying diagram. The mixture of air and respired $CO_2$ was forced down the central tube whence it rose slowly in small bubbles through the spiral tube. The barium carbonate precipitate fell to the bottom of the absorption tube and did not obstruct the continued passage of gas through

the solution. By regulating the flow of gas to a slow, steady stream a very effective absorption was attained. When heavy concentrations of carbon dioxide were encountered two or more units were connected together thus assuring that no appreciable carbon dioxide gas escaped absorption. An even current of gas through the absorption tubes could be maintained by applying a slight suction at the end of the entire system. This also prevented any rise of pressure in the system which might unseat the stoppers.

Titration of the residual barium hydroxide was carried out directly in the absorption vessel against standard HCl solution with phenolphthalein as an indicator and using the glass spiral as a stirrer. The titration procedure employed was similar to that used by GURJAR (**15**). MARTIN and GREEN (**18**) have shown that spiral absorbers are a very effective means for absorbing carbon dioxide and also that titrating directly in the absorption vessel in the presence of suspended barium carbonate does not cause a loss of carbon dioxide through the decomposition of the carbonate by the acid added during the titration.

In the studies previously reported from this laboratory the $CO_2$ of respiration was allowed to accumulate for four days and was then removed and determined. This total was divided by four to give the average per 24 hours in which form it was recorded. The same procedure was followed in the researches here reported, and in addition a parallel series was provided from which the respired $CO_2$ was removed at the end of each 24-hour period for four consecutive days and the total divided by four to give the average per 24 hours. Both sets of data are recorded in the following tables. It will be noted that daily aspiration resulted invariably in larger average yields of respired $CO_2$, but the two sets of data yield curves of the same general shape if corrected for the differences in quantities involved. Accordingly it appears that there is no singular advantage attached to one technique over the other in determining the effect of the several variables studied upon the *relative* level of respiration.

Continuous aspiration was not attempted because of the obvious difficulties involved in preventing changes in the moisture content of the grain in consequence of the continuous passage of a current of air through it.

## Experimentation

### RELATION BETWEEN MOISTURE CONTENT AND RESPIRATION OF OATS

Four varieites of clean, sound oats from the 1933 crop grown on the University Farm, St. Paul, Minnesota, were obtained for the determination of respiration rates at various moisture levels. In this study an attempt was made to obtain sufficient data so that the average value reported would give a correct indication of the rate of respiration of the grain under the conditions noted. Thus each value recorded for the four moisture levels

represented the average of at least eight separate determinations. It is obviously impractical to report all of these detailed data. Therefore, in addition to the use of average values for individual samples, further condensation is accomplished by reporting only the average respiratory rate values of several samples.

Table I and figure 2 indicate the average rate of respiration as a function

TABLE I

AVERAGE RATE OF RESPIRATION OF FOUR VARIETIES OF OATS

| CARBON DIOXIDE RESPIRED PER 24 HOURS PER 100 GM. DRY MATTER | | |
|---|---|---|
| MOISTURE | DAILY ASPIRATIONS | ASPIRATED AT END OF FOUR DAY PERIOD |
| % | *mg.* | *mg.* |
| 11.0 | 0.2 | 0.1 |
| 13.0 | 0.7 | 0.2 |
| 15.0 | 2.8 | 1.4 |
| 17.0 | 13.5 | 8.0 |

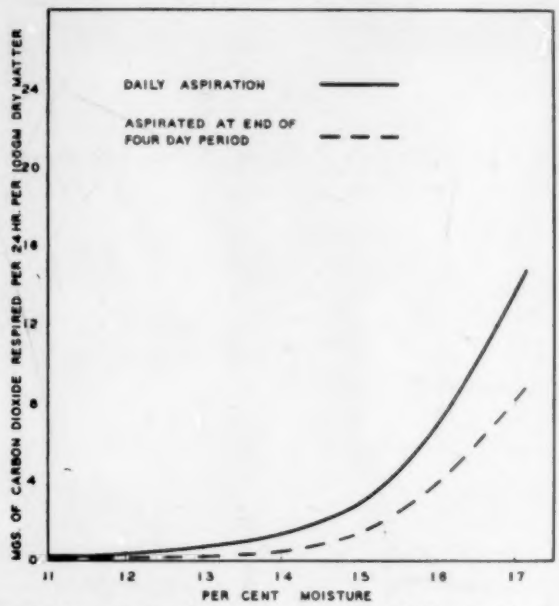



FIG. 2. Respiration of oats showing the retarding influence of accumulated carbon dioxide on respiratory rate.

of moisture content in the instance of three varieties of oats. The values reported in this and subsequent tables were obtained by plotting graphs of the individual respiration curves. By interpolation of these curves carbon

dioxide respired (in milligrams) at the moisture levels of 11, 13, 15 and 17 per cent. was computed. When water was added to the dry samples of grain it was not possible to secure exactly the percentage of moisture desired; it was seldom, however, that the actual moisture deviated from the desired level by more than 0.5 per cent.

Table I and figure 2 present typical examples of the increase in rate of respiration of grain as the moisture content exceeds 15 per cent. Likewise, the retarding influence of accumulated carbon dioxide on the respiratory rate is plainly indicated. LARMORE, CLAYTON, and WRENSHALL (**17**) also found that the respiration rate in continuously aspirated grain was higher than when discontinuous aspiration was applied. With discontinuous aspiration the rate was dependent upon the free space in the container.

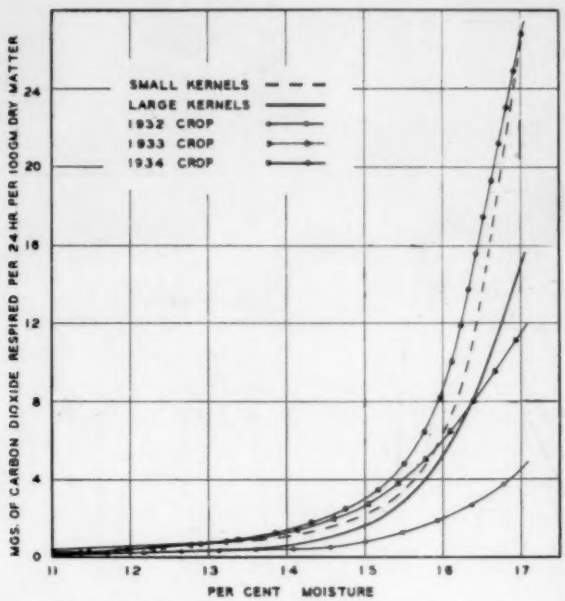



FIG. 3. Respiration of barley showing the influence of kernel size and the differences between crop seasons.

The average respiratory rate of all samples of oats compared with other grains is shown in table IX and figure 4. These values represent the accumulated carbon dioxide after a four-day undisturbed storage period and by computing the rate on the basis of milligrams of carbon dioxide respired per twenty-four hours per one hundred grams of dry matter.

### RELATION BETWEEN MOISTURE CONTENT AND RESPIRATION OF BARLEY

The respiratory rate of barley at various moisture levels was determined in the same manner as that just described for oats. In order to compare,

from year to year, the respiration rate of barley grown in the same locality and on the same soil a further investigation was made by obtaining samples of barley grown at University Farm during the crop seasons of 1932, 1933, and 1934. In table II and figure 3 the data from the three crop years are recorded separately and it appears that the 1933 and 1934 crop samples behaved similarly, but that the respiration of the 1932 crop was considerably lower. This may be attributed in part at least, to the fact that the 1933 and 1934 crop growing periods were extremely dry and, hence, the barley kernels were of smaller size and shriveled, while 1932 was a year of normal rainfall in the locality where the barley was grown which tended to produce more plump kernels. The viability of the barley samples for the three years was nearly the same. The relation between kernel size and rate of respiration will be discussed later on in this paper. It is evident from the data presented in table II that at the same moisture level there is a variation in the likelihood of barley heating during storage, as crop conditions change from year to year.

TABLE II

COMPARISON OF THE RATE OF RESPIRATION OF BARLEY FOR THE YEARS 1932, 1933 AND 1934

| MOISTURE | YEAR | $CO_2$ RESPIRED PER 24 HR. PER 100 GM. DRY MATTER | |
|---|---|---|---|
| | | DAILY ASPIRATION | ASPIRATED AFTER 4 DAYS |
| % | | *mg.* | *mg.* |
| 11.0 | 1932 | 0.1 | 0.05 |
| 11.0 | 1933 | 0.1 | 0.05 |
| 11.0 | 1934 | 0.3 | 0.20 |
| 13.0 | 1932 | 0.3 | 0.1 |
| 13.0 | 1933 | 0.7 | 0.4 |
| 13.0 | 1934 | 0.7 | 0.4 |
| 15.0 | 1932 | 0.8 | 0.5 |
| 15.0 | 1933 | 2.6 | 2.0 |
| 15.0 | 1934 | 3.0 | 2.4 |
| 17.0 | 1932 | 4.9 | 2.8 |
| 17.0 | 1933 | 11.6 | 9.1 |
| 17.0 | 1934 | 26.4 | 16.0 |

The average respiratory rate of all samples of barley is reported in table IX and figure 4. Compared to other grains, barley is intermediate in its rate of respiration.

### RELATION BETWEEN MOISTURE CONTENT AND RESPIRATION OF RYE

The rye samples included in this study evidenced a low respiratory rate as indicated by the values recorded in table III and shown graphically in figure 4. There should be a correspondingly reduced hazard of rye heating

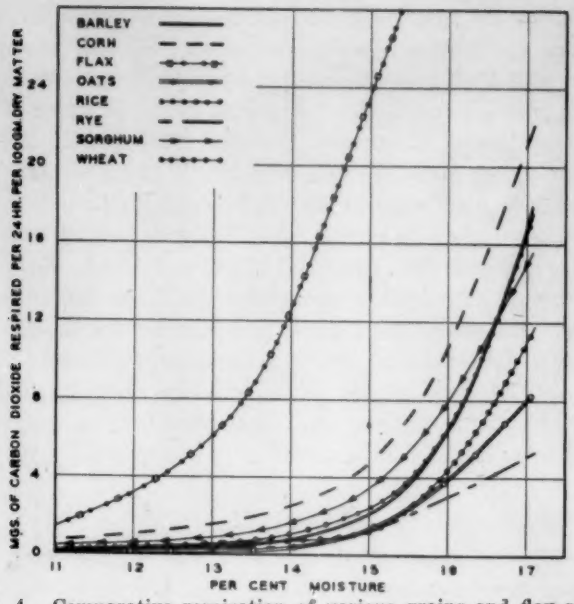



FIG. 4. Comparative respiration of various grains and flax seed.

during storage when harvesting conditions are normal. The small germ area of rye compared with the total area of the kernel, and the relatively smooth surface of the rye kernel, which is not conducive to micro-organism growth may both contribute to the low rate of respiration of this cereal.

### RESPIRATION OF FLAXSEED

Table IV and figure 4 indicate that flaxseed has the highest respiratory rate of any seed thus far reported. This is not unexpected in view of the high oil content of flax seed. The known fact that the embryo cells of seeds contain a high proportion of lipids and likewise respire much more vigor-

TABLE III

AVERAGE RATE OF RESPIRATION OF RYE

| MOISTURE | CARBON DIOXIDE RESPIRED PER 24 HR. PER 100 GM. DRY MATTER | |
|---|---|---|
| | DAILY ASPIRATIONS | ASPIRATED AT END OF 4-DAY PERIOD |
| % | *mg.* | *mg.* |
| 11.0 | 0.2 | 0.1 |
| 13.0 | 0.5 | 0.3 |
| 15.0 | 2.0 | 1.2 |
| 17.0 | 9.3 | 5.1 |

TABLE IV

AVERAGE RATE OF RESPIRATION OF FLAX

| MOISTURE | CARBON DIOXIDE RESPIRED PER 24 HOURS PER 100 GRAMS DRY MATTER | |
|---|---|---|
| | DAILY ASPIRATIONS | ASPIRATED AT END OF 4-DAY PERIOD |
| % | *mg.* | *mg.* |
| 11.0 | 2.5 | 1.4 |
| 13.0 | 9.2 | 6.1 |
| 15.0 | 48.4 | 23.9 |
| 17.0 | 159.3 | 67.4 |

ously than endosperm cells would suggest that oil seeds such as flax might be expected to have a higher respiratory level than farinaceous material.

Another significant feature of this situation is the probable distribution of the moisture in an oil seed. When approximately 40 per cent. of the dry substance of a flaxseed is an oil that is essentially immiscible with water, it must follow that the actual moisture content of the hygroscopic substance present will be higher than in a farinaceous endosperm comprised largely of hydrophilic materials. Thus, for example, if we assume that a flaxseed contains 40 per cent. of oil (dry matter basis) that is immiscible with water, then an apparent moisture content of 10 per cent. of the entire seed might mean an actual content of water in the hydrophilic substances of approximately 16.5 per cent. Applying such a correction to the data recorded in table IX and figure 4 would tend to bring the flaxseed values in line with those of the cereal grains. Of course the problem is not as simple as is suggested by such a calculation, but the issue raised here may be significant.

### RELATION OF RESPIRATION RATE TO THE GRAIN GRADE

To determine the rate of respiration of samples of barley, oats, and rye such as are actually being handled and stored in the grain trade, composite samples of different grades of these three grains were obtained from the State Grain Inspection Department in Minneapolis. The Federal standards recognize a number of numerical grades of grain based on such factors as: limits of foreign material, test weight, damaged kernels, moisture, etc. Four grades of barley, three grades of oats, and three grades of rye were thus procured. Since the samples were composite ones no basis for the differentiation in grade can be given. In all cases, however, the samples were composed of sound grain.

The results of the study using these samples are recorded in tables V, VI, and VII. All grades show the same rapid rise in production of carbon dioxide when the moisture level is above 15 per cent. There is, moreover,

## TABLE V

COMPARISON OF THE RATE OF RESPIRATION OF FOUR GRADES OF BARLEY AT THE SAME MOISTURE LEVEL. INTERPOLATED VALUES

| MOISTURE | GRADE | $CO_2$ RESPIRED PER 24 HR. PER 100 GM. DRY MATTER | |
|---|---|---|---|
| | | DAILY ASPIRATION | ASPIRATED AFTER 4 DAYS |
| % | | *mg.* | *mg.* |
| 11.0 | No. 2 | 0.4 | 0.2 |
| 11.0 | No. 3 | 0.2 | 0.1 |
| 11.0 | Sample | 0.1 | 0.1 |
| 11.0 | Feed | 0.2 | 0.2 |
| 13.0 | No. 2 | 0.7 | 0.4 |
| 13.0 | No. 3 | 0.4 | 0.3 |
| 13.0 | Sample | 0.4 | 0.2 |
| 13.0 | Feed | 1.0 | 0.6 |
| 15.0 | No. 2 | 2.3 | 1.8 |
| 15.0 | No. 3 | 1.6 | 1.0 |
| 15.0 | Sample | 1.5 | 0.8 |
| 15.0 | Feed | 3.0 | 2.0 |
| 17.0 | No. 2 | 12.7 | 9.4 |
| 17.0 | No. 3 | 19.0 | 13.3 |
| 17.0 | Sample | 8.4 | 5.4 |
| 17.0 | Feed | 19.9 | 18.5 |

## TABLE VI

COMPARISON OF THE RATE OF RESPIRATION OF THREE GRADES OF OATS AT THE SAME MOISTURE LEVEL. INTERPOLATED VALUES

| MOISTURE | GRADE | $CO_2$ RESPIRED PER 24 HR. PER 100 GM. DRY MATTER | |
|---|---|---|---|
| | | DAILY ASPIRATION | ASPIRATED AFTER 4 DAYS |
| % | | *mg.* | *mg.* |
| 11.0 | No. 3 | 0.2 | 0.1 |
| 11.0 | No. 4 | 0.2 | 0.1 |
| 11.0 | Sample | 0.2 | 0.1 |
| 13.0 | No. 3 | 0.4 | 0.2 |
| 13.0 | No. 4 | 0.4 | 0.2 |
| 13.0 | Sample | 0.5 | 0.3 |
| 15.0 | No. 3 | 0.9 | 0.6 |
| 15.0 | No. 4 | 1.5 | 0.8 |
| 15.0 | Sample | 2.7 | 1.8 |
| 17.0 | No. 3 | 21.1 | 8.5 |
| 17.0 | No. 4 | 12.6 | 7.0 |
| 17.0 | Sample | 18.0 | 7.7 |

TABLE VII

COMPARISON OF THE RATE OF RESPIRATION OF THREE GRADES OF RYE AT THE SAME MOISTURE LEVEL. INTERPOLATED VALUES

| MOISTURE | GRADE | $CO_2$ RESPIRED PER 24 HR. PER 100 GM. DRY MATTER | |
|---|---|---|---|
| | | DAILY ASPIRATION | ASPIRATED AFTER 4 DAYS |
| % | | *mg.* | *mg.* |
| 11.0 | No. 1 | 0.2 | 0.1 |
| 11.0 | No. 2 | 0.1 | 0.1 |
| 11.0 | No. 3 | 0.2 | 0.1 |
| 13.0 | No. 1 | 0.4 | 0.2 |
| 13.0 | No. 2 | 0.4 | 0.2 |
| 13.0 | No. 3 | 0.7 | 0.4 |
| 15.0 | No. 1 | 1.7 | 1.0 |
| 15.0 | No. 2 | 2.6 | 1.7 |
| 15.0 | No. 3 | 2.3 | 1.3 |
| 17.0 | No. 1 | 6.7 | 5.2 |
| 17.0 | No. 2 | 9.9 | 5.7 |
| 17.0 | No. 3 | 14.0 | 6.1 |

considerable variation in the quantity of carbon dioxide evolved by the different grades at the same moisture level, but the variation does not appear to be related to the grade.

### RELATION OF THE RELATIVE PLUMPNESS OF THE BARLEY KERNEL TO THE RATE OF RESPIRATION

Two samples of barley which differed greatly in kernel size were obtained from one of the malting plants in Minneapolis. These samples are here designated as large and small size kernels; to the maltster they are known as "malting size" and "needles" respectively. The 1000 kernel weights were 39.2 and 17.2 grams respectively, and germination was 94 per cent. in each case. Table VIII shows the respiration data of these two lots of barley and the same data are shown graphically in figure 3. The decided differences in the rate of respiration of large and small kernels from the same lots of barley substantiates what has previously been contended; namely, that for equal weights of different sized kernels the smaller kernels have the highest respiration rate. BAILEY and GURJAR (**4**) found this to be true of wheat kernels.

### CHANGE IN THE DIELECTRIC PROPERTIES OF OATS AND BARLEY WITH CHANGE IN THE MOISTURE CONTENT OF THE GRAIN

It is generally recognized that the water present in a hydrogel exists in several states or conditions. As suggested by FOOTE and SAXTON (**11**) these

TABLE VIII

RATE OF RESPIRATION OF BARLEY

| MOISTURE | CARBON DIOXIDE RESPIRED PER 24 HR. PER 100 GM. DRY MATTER | |
|---|---|---|
| | LARGE SIZE KERNELS | |
| | DAILY ASPIRATION | ASPIRATED AT END OF 4 DAY PERIOD |
| % | *mg.* | *mg.* |
| 11.49 | 0.26 | 0.13 |
| 13.07 | 0.40 | 0.32 |
| 15.05 | 1.68 | 1.32 |
| 17.07 | 14.99 | 13.89 |
| | SMALL SIZE KERNELS | |
| 11.00 | 0.21 | 0.14 |
| 12.95 | 0.71 | 0.48 |
| 15.35 | 2.95 | 2.05 |
| 17.02 | 26.40 | 22.26 |

states may be described as free, capillary, and combined. GORTNER (**14**) has ably discussed the free and bound states of water in biological material, and the differences in physical properties between these two states. It has recently developed that indirect physical measurements were becoming increasingly useful and significant in distinguishing between these states. One such means involves the observation of changes in capacitance of such materials as cereal grains as a function of the amount, and hence of the state of water present.

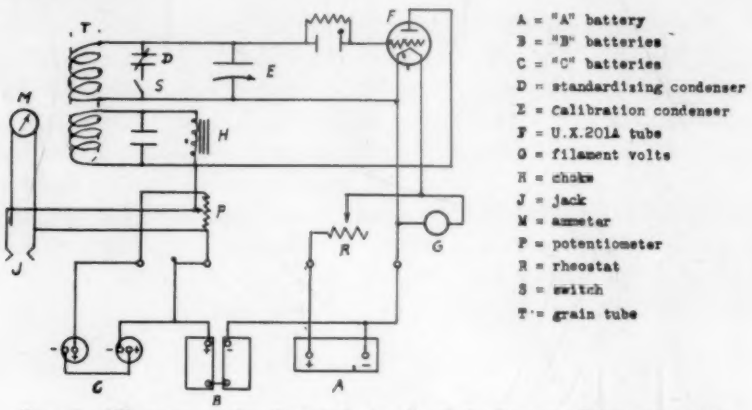



FIG. 5. Diagram of the electrical circuit of the BURTON-PITT apparatus.

The BURTON-PITT (**8**) device which was available in this laboratory is adapted to this end. It makes use of the high frequency circuit diagrammed

in figure 5. When a tube containing grain is introduced into the electrical field produced by the current at the position marked T in the diagram, a change occurs in the current flowing through the ammeter, which can be observed and recorded.

Observations were accordingly made upon one series each of oats and barley samples, in which the sole variable was moisture content. The rate of respiration, and the milliammeter reading as observed with the BURTON-PITT device are recorded in table X. While these two sets of values are not

## TABLE IX

COMPARISON OF THE RATE* OF RESPIRATION OF GRAINS AT FOUR MOISTURE LEVELS. RESULTS EXPRESSED IN MGS. OF $CO_2$ PER 24 HR. PER 100 GM. DRY MATTER

| KIND OF GRAIN | PERCENTAGE MOISTURE | | | |
|---|---|---|---|---|
| | 11 | 13 | 15 | 17 |
| | *mg.* | *mg.* | *mg.* | *mg.* |
| Barley | 0.1 | 0.4 | 2.1 | 16.7 |
| Corn† | 0.7 | 1.5 | 4.7 | 21.9 |
| Flax | 1.4 | 6.1 | 23.9 | 67.4 |
| Oats | 0.1 | 0.2 | 1.4 | 8.0 |
| Rice‡ | 0.2 | 0.5 | 2.4 | 16.5 |
| Rye | 0.1 | 0.3 | 1.2 | 5.1 |
| Sorghum§ | 0.5 | 0.9 | 3.5 | 15.0 |
| Wheat§§ | 0.2 | 0.5 | 1.2 | 11.0 |

* Values obtained by interpolation and extrapolation of data.
† Average values from (3).
‡ Values from (5b).
§ Values from (10).
§§ Values from (4).

## TABLE X

AVERAGE RATE OF RESPIRATION AND BURTON-PITT MOISTURE VALUES FOR ALL SAMPLES OF OATS AND BARLEY

| MOISTURE | OATS | | BARLEY | |
|---|---|---|---|---|
| | $CO_2$ PER 24 HR. PER 100 GM. | BURTON-PITT VALUES | $CO_2$ PER 24 HR. PER 100 GM. | BURTON-PITT VALUES |
| % | *mg.* | $\frac{MA \times 100}{wt.\ of\ grain}$ | *mg.* | $\frac{MA \times 100}{wt.\ of\ grain}$ |
| 11.0 | 0.2 | 0.55 | 0.2 | .49 |
| 13.0 | 0.5 | 0.63 | 0.6 | .54 |
| 15.0 | 1.9 | 0.82 | 1.6 | .69 |
| 17.0 | 11.3 | 1.09 | 13.7 | .84 |

exactly parallel, they approach that relationship. As indicated by GORTNER (13) the sharp acceleration of respiration at certain levels of moisture content may be the consequence of bound water relationships, and it is also probable that the same is true of the observed capacitance values.

While the data here recorded do not make certain facts abundantly clear, it has appeared from extensive experience with the BURTON-PITT device that the milliammeter readings are a function of grain properties other than moisture content. In fact the same variables which affect respiratory level of grain likewise tend to influence the milliammeter readings in the same direction and to much the same degree. It will require additional research to establish this fact, but it appears to be a safe prediction that such readings as are secured by this instrument will constitute more useful criteria of the keeping qualities of grain in storage than any single chemical or physical characteristic of which we are aware.

The exponential types of curve which expresses the relation between the moisture content of corn and respiratory rate suggests that log ($CO_2$ respired) might become a linear function of moisture content. Letting $y$ = $CO_2$ respired per 100 gm. dry matter in each 24 hours, and $x$ = moisture content, log $y$ was plotted against $x$ and this turned out to be a curve rather than a straight line. This made it evident that the values for $y$ must be corrected, possibly by a constant $c$. The value for $c$ was computed through the use of the equation $y = ae^{bx} + c$. and was found to be $c = 0.672$. Accordingly when all values for $y$ were corrected by subtracting 0.672 and log $(y–c)$ was plotted against $x$ a straight line resulted. For average corn of all varieties each increase in moisture content of 1 per cent. was accompanied by an increase in the value of log $(y - 0.672) = 0.350$. In a similar calculation applied to the data resulting from the study of sound hard spring wheat $c$ = 0.4926 and the average increase in the value log $(y - 0.4926) = 0.63$ per 1 per cent. increase in moisture content.

## Summary

Curves which represent the relationship between moisture content and respiratory rate of stored cereals are expressed mathematically by an exponential formula which approaches a linear form when the logarithm (base 10) of the rate of respiration is recorded against the arithmetical progression of moisture content. For the earlier researches on wheat the equation $y = ae^{bx} + c$ in which $x$ = moisture content and $y = CO_2$ respired, and $c$ = 0.4926 serves to express the relation between these variables between the limits of 11 and 17 per cent. of moisture. For corn or maize, the value of $c$ in the equation becomes 0.672.

Rice and barley behave much like wheat in these particulars. Oats display less acceleration of respiration per unit increase in moisture content than do rice, barley, and wheat, while the rye samples studied deviated still more widely in this respect.

No significant correlation between market grade of barley and oats, and their rate of respiration at unit levels of moisture content appeared in the

instance of the samples included in these studies. When shriveled or small barley kernels were compared with plump or large kernels, the former registered a distinctly higher level of respiration. This relationship had previously been observed in the instance of wheat.

Flax seed respires much more vigorously than the cereals at all levels of moisture content in the range studied (11–17 per cent.).

It is suggested that this difference may result from the fact that the actual moisture content of the hydrophylic substances in the flax seed may be substantially higher than is suggested by the percentage of moisture in the entire seed.

Relative capacitance of cereal grains as measured by means of the BURTON-PITT instrument may be more highly correlated with rate of respiration than any other single chemical or physical determination heretofore employed as a basis of prediction.

The writer wishes to acknowledge the technical assistance of J. J. MARTIN and J. S. SHELLENBARGER in this study.

UNIVERSITY OF MINNESOTA
ST. PAUL, MINNESOTA

## LITERATURE CITED

1. AGRONOMOFF, E. A. Temperature changes of stored grain. Scientific Institute of Cereal Research. Moscow. U.S.S.R. 1931.
2. BAILEY, C. H. The handling and storage of spring wheat. Jour. Amer. Soc. Agron. **9**: 275–281. 1917.
3. ————. Respiration of shelled corn. Minnesota Agr. Exp. Sta. Tech. Bull. 3. 1921.
4. ————, and GURJAR, A. M. Respiration of stored wheat. Jour. Agr. Res. **12**: 685–713. 1918.
5. ————, and ————. Respiration of cereal plants and grains.
   (a) II. Respiration of sprouted wheat. Jour. Biol. Chem. **44**: 5–7. 1920.
   (b) III. Respiration of rice paddy and milled rice. Jour. Biol. Chem. **44**: 9–12. 1920.
   (c) IV. Respiration of frosted wheat plants. Jour. Biol. Chem. **44**: 13–15. 1920.
   (d) V. Note on the respiration of plants infected with stem rust. Jour. Biol. Chem. **44**: 17–18. 1920.
6. BAKKE, A. L. and NOECKER, N. L. The relation of moisture to respiration and heating in stored oats. Iowa Agr. Exp. Sta. Res. Bull. 165. 1933.
7. BROWNE, C. A. The spontaneous heating and ignition of hay and other agricultural products. Science n.s. **77**: 223–229. 1933.

8. Burton, E. F. and Pitt, Arnold. A new method of conductivity measurements by means of an oscillating valve circuit. Phil. Mag. (Series 7) **5**: 939–943. 1928.
9. Coleman, D. A. and Fellows, H. C. Hygroscopic moisture of cereal grains and flaxseed exposed to atmospheres of different relative humidity. Cereal Chem. **2**: 275–287. 1925.
10. ————, Rothgeb, B. E. and Fellows, H. C. Respiration of sorghum grains. U.S.D.A. Tech. Bull. 100. 1928.
11. Foote, H. W., and Saxton, B. The effect of freezing on certain inorganic hydrogels. Jour. Amer. Chem. Soc. **38**: 588. 1916.
12. Gilman, J. C. and Barron, D. H. Effect of molds on temperature of stored grain. Plant Physiol. **5**: 565–573. 1930.
13. Gortner. R. A. Outlines of biochemistry. p. 242. John Wiley and Sons, Inc. New York. 1929.
14. ————. Annual review of biochemistry. **1**: 21–51. 1932.
15. Gurjar, A. M. The adaptation of Truog's method for the determination of carbon dioxide to plant respiration studies. Plant World **20**: 288–293. 1917.
16. James, L. H., Rettger, L. F., and Thom, Charles. Microbial thermogenesis II. Heat production in moist organic materials with special reference to the part played by microörganisms. Jour. Bact. **15**: 117–141. 1928.
17. Larmore, R. K., Clayton, J. S., and Wrenshall, C. L. A study of the respiration and heating of damp wheat. Canadian Jour. Res. **12**: 627–645. 1935.
18. Martin, W. McK. and Green, J. R. Determination of carbon dioxide in continuous gas streams. Ind. and Eng. Chem. Anal. Ed. **5**: 114–118. 1933.
19. Rozsa, T. A. Drinking habits of the wheat berry. The Northwestern Miller, **184**: 73–74. 1935.
20. Skovholt. Oscar and Bailey, C. H. Some new facts about molds and bread. Minnesota Agr. Exp. Sta. Bull. 296. 1933.
21. Swanson, C. O. Some factors involved in damage to wheat quality. Cereal Chem. **11**: 173–199. 1934.
22. Wilson, R. E. Humidity control by means of sulphuric acid solution, with critical compilation of vapor pressure data. Ind. and Eng. Chem. **13**: 326–331. 1921.

# EFFECT OF LIGHT INTENSITY ON THE DEVELOPMENT OF THE PHOTOSYNTHETIC MECHANISM[1]

MARSTON C. SARGENT

(WITH THREE FIGURES)

## Introduction

LUBIMENKO (**13**) was the first to observe differences in assimilatory activity between trees which ordinarily thrive only in the shade and those which thrive only in sunny places. He found that the shade species had a higher rate of photosynthesis than the sun species at low light intensities. At high intensities, however, the photosynthesis of the sun species was accelerated until it surpassed that of the shade species. He also observed that chloroplasts of the sun species, although as numerous as those of shade species, were less green and hence, he concluded, contained less chlorophyll. BOYSEN-JENSEN (**2**) and LUNDEGÅRDH (**14**), using herbaceous plants, confirmed LUBIMENKO's observations on rates of photosynthesis at high and low light intensities. JOHANNSON (**12**) reported in the case of a fern, and MONTFORT (**15**) in Cladophora that the assimilation of shade species was partially suppressed in direct sunlight. That these differences were not due in all cases to genetic factors was shown by the work of BOYSEN-JENSEN and MÜLLER (**3**), who found that leaves from the sunny and shady sides of a beech tree exhibited the same differences in rates of assimilation that LUBIMENKO observed. Similar phenomena, according to HARDER (**11**), occur in ivy leaves.

In all these cases, many external factors besides light intensity varied systematically with the environment. In order to exclude such factors, the experiments reported in this paper were carried out with the unicellular green alga *Chlorella pyrenoidosa*, which can be grown in pure culture under uniform conditions of temperature, carbon dioxide pressure, and concentration of nutrients, in light of which the wave-length distribution is maintained constant while intensity is varied at will. Using this single species of plant, grown under two selected intensities of illumination, measurements were made of (1) the maximum rate of assimilation, (2) the variation of rate of assimilation with light intensity, and (3) chlorophyll content. These measurements make it possible to revaluate the observations of earlier workers and to clarify certain characteristics of the photosynthetic mechanism which have not been emphasized in recent discussions.

[1] These experiments were conducted at the California Institute of Technology, Pasadena, and the Carnegie Coastal Laboratory, Carmel, California.

## Methods

A description of the general method of cultivating Chlorella is to be found in a paper by GAFFRON (9). In the present experiments, two glass-bottomed running-water culture baths illuminated from below were used side by side in a dark room. In order to obtain "sun" and "shade" conditions, one bath was illuminated by four internally frosted incandescent lamps, burned base down with their tips 10 cm. from the bottom of the bath; the other was illuminated by a single 40-watt bulb at 17 cm. The use of bulbs of the same rating insured that the spectral composition of the light was identical, and a Weston photoelectric cell showed that the illumination on the bottom of the sun bath was seven times that of the shade bath. The total energy in the visible region falling on the bottom of the sun bath was about the same as would fall on the same area in moderately bright sunlight, but because of the difference in wave-length distribution between sunlight and the light from an incandescent filament, the two sources could not be expected to have identical effects on the complex of biological processes under discussion.

The two intensities specified were selected after a number of preliminary experiments. At intensities lower than that in the shade bath, growth was excessively slow. At intensities higher than that in the sun bath, the cultures were overheated. The intensities used were sufficiently far apart to engender marked quantitative differences in the characteristics of the algae which were of the same nature as the differences observed by earlier workers between plants in sunny or shady places.

Assimilation was measured manometrically (9) at 25° C. in Knop's solution saturated with five per cent. carbon dioxide in air. The highest illumination used was that from a closely spaced row of 100-watt bulbs at 20 cm. which is roughly comparable with bright sunlight. This intensity was reduced at need by fastening Wratten neutral filters to the bottoms of the manometer vessels, whose tops and sides were covered with brass foil. The filters were calibrated, while immersed, with a photoelectric cell, and were found to have their rated transmissions, except for the "100 per cent.," which had an actual transmission of only 93 per cent.

The concentration of chlorophyll was estimated by extracting a sample of cells with hot methyl alcohol and measuring the transmission of the extract in light of wave length 6598.953 Å from a neon tube, using a visual spectrophotometer (see EMERSON and ARNOLD (6), p. 192, for full description and determination of the standard extinction coefficient). ARNOLD and KOHN (1) found that chlorophyll from three different plants (Chlorella, Equisetum, and spinach) had the same standard extinction coefficient at this wave length. This means that the ratio of chlorophyll *a* to chlorophyll *b* is the same for these three plants within the limits of accuracy of the method of estimation, and affords no grounds for assuming that the ratio is,

in general, labile. In the present series of experiments, attempts to measure the ratio of *a* to *b* by measuring the absorption at two wave lengths failed to give reliable results.

Rates of assimilation and chlorophyll content in the present work are referred to a standard volume of cells as packed by centrifuging. When a suspension of Chlorella is centrifuged in a haematocrit for lengthening periods or at increasing velocities, the readings are found to approach a minimum value asymptotically. At 3500 r.p.m. (radius 20 cm.) and 10 minutes, small variations in velocity and time made an inappreciable difference in the volume determinations. One hundred cubic millimeters of cells have a dry weight of approximately 20 mg.

## Experimental results

Early in the investigation it became apparent that while the cultures were growing, the nature of the cells of which they were composed changed progressively from day to day. A sample of cells from a freshly inoculated, thinly populated culture differed, with respect to the characteristics studied, from an old densely populated culture. Three factors in the environment can be identified as contributing to this progressive change. First, as a culture grows, the concentration of nutrient falls. Second, the average intensity of light within the culture decreases. Third, the average spectral distribution of light within the culture changes because of the selective absorption of a Chlorella suspension. Hence it is meaningless to compare a single sun culture with a single shade culture at random. Because they grow so much faster in the sun bath than in the shade bath, cultures of the same chronological age are not comparable. More nearly satisfactory is the comparison of cultures of the same population density, at least when this is low. As will be shown below, however, a suspension of sun cells transmits more light than a suspension of shade cells having the same cell concentration and this affects both the average intensity in the culture and its spectral distribution. But if cultures at all stages of the growth cycle are studied, it is found that whatever differences were present initially, persist in some degree to the end of the cycle. Determinations of the maximum rate of photosynthesis and the chlorophyll content of cells from such series of cultures were made and are here reported.

It should be emphasized that in pure cultures of *C. pyrenoidosa* the cells do not ordinarily die. An old culture whose population has increased a thousandfold from its original size will contain no cells which, under microscopic examination, are recognizably dead or even moribund. Hence changes found by observing a sample of cell suspension can not be ascribed simply to the presence of a varying proportion of dead cells.

The fact that plants growing in the shade are greener than those growing

under high illumination can often be noted in the field, and the degree of greenness has been correlated with chlorophyll content by a number of investigators (**13, 17, 20**). The chlorophyll content of cells cultivated in the sun bath is approximately half that of shade cells. Table I gives the results

TABLE I

THE CONCENTRATION* OF CHLOROPHYLL IN CELLS FROM YOUNG SUN AND SHADE CULTURES

| | SUN CULTURES | SHADE CULTURES |
|---|---|---|
| | *gm.* | *gm.* |
| | 0.026 | 0.071 |
| | 0.036 | 0.071 |
| | 0.032 | 0.065 |
| | 0.031 | 0.055 |
| | 0.033 | |
| | 0.034 | |
| | 0.037 | |
| | 0.037 | |
| | 0.035 | |
| Mean | 0.0334 | 0.0655 |
| Ratio of means | $\frac{0.51}{1.00}$ | |

* Grams of chlorophyll per gram of cells (dry weight).

of a number of determinations expressed as grams of chlorophyll in one gram of cells (dry weight). WARBURG'S data (**19**) show that under the culture conditions he specified which were similar to, but not identical with, the present ones, "Tageslichtzellen" had a chlorophyll content of 2.6 per cent. of the dry weight, while "Schattenzellen" had a content of 4.0 per cent.

TABLE II

CONCENTRATION OF CHLOROPHYLL IN CELLS FROM CULTURES OF DIFFERENT AGES

| SUN CULTURES | | | SHADE CULTURES | | |
|---|---|---|---|---|---|
| AGE OF CULTURE | POPULATION DENSITY* | CHLOROPHYLL CONTENT† | AGE OF CULTURE | POPULATION DENSITY* | CHLOROPHYLL CONTENT† |
| *days* | *mm.*$^3$ | *gm.* | *days* | *mm.*$^3$ | *gm.* |
| 3 | 0.11 | 0.033 | 7 | 0.068 | 0.071 |
| 4 | 0.37 | 0.034 | 14 | 0.23 | 0.065 |
| 6 | 1.1 | 0.037 | 23 | 0.65 | 0.078 |
| 8 | 1.5 | 0.045 | 30 | 0.95 | 0.073 |
| 10 | 1.7 | 0.049 | 37 | 1.3 | 0.063 |
| | | | 44 | 1.6 | 0.062 |
| | | | 49 | 1.7 | 0.069 |

* Mm.$^3$ cells per cubic centimeter.
† Grams of chlorophyll per gram of cells (dry weight).

As a sun culture grows older, the chlorophyll content of a unit volume of cells increases appreciably, while in a shade culture, there is no significant change. Table II shows that while the difference between sun and shade cells is diminished in this way, it is still perfectly apparent at all stages of the growth cycle. The increase in chlorophyll content in the sun cultures can be ascribed to decrease of the average light intensity inside the cultures. Changes in the spectral distribution of the light may also play a part. From the work of EMERSON (5) and FLEISCHER (8) we should expect depletion of nutrients to act in the opposite direction, so we must conclude that this factor is either not operative, or that its effect is obscured by the light effect. Because the chlorophyll content of shade cultures is constant for all densities of population, it is possible to conjecture that at very low light intensities, chlorophyll content changes very slowly with change of intensity and approaches asymptotically a maximum value.

The maximum rate of assimilation at 25° C. that is, the rate measured at such a carbon dioxide concentration and light intensity that an increase in either of these factors (or both of them) causes no increase in assimilation is, by definition, the rate of the Blackman reaction. In cells from young sun cultures this process is about one and a half times as fast as in cells from young shade cultures. Table III shows that there is considerable

TABLE III

MAXIMUM RATE* OF PHOTOSYNTHESIS OF CELLS FROM SUN AND SHADE CULTURES

| | SUN CULTURES | SHADE CULTURES |
|---|---|---|
| | $mm.^3$ $O_2$ | $mm.^3$ $O_2$ |
| | 64 | 29 |
| | 56 | 42 |
| | 45 | 36 |
| | 53 | 39 |
| | 50 | 45 |
| | 65 | 32 |
| | 55 | |
| Mean | 55.4 | 37.2 |
| Ration of means | $\frac{1.5}{1.0}$ | |

* Mm.³ oxygen per hour per mm.³ cells.

variation in this rate from culture to culture, but that a real difference exists between sun and shade cells. In all of the literature on Chlorella photosynthesis, no rates are reported as high as those given in table III for sun cells, and it is probable that the cells have always been grown at light intensities lower than that in the sun bath of the present experiments.

This difference between sun and shade cells is qualitatively like that

observed by previous workers (**2, 13**). The numerical value of the ratio depends on the exact environmental conditions specified and hence is not suitable for analytical treatment. As the difference in assimilatory rates is opposite in sense to the difference in chlorophyll content, the rate of the Blackman reaction is not a function of the chlorophyll content.

With increasing density of population the maximum rate of assimilation of both sun and shade cells decreases as shown in table IV. The rate of

TABLE IV

MAXIMUM RATE OF PHOTOSYNTHESIS OF CELLS FROM CULTURES OF DIFFERENT AGES

| SUN CULTURES | | | SHADE CULTURES | | |
|---|---|---|---|---|---|
| AGE OF CULTURE | POPULATION DENSITY | MAXIMUM PHOTOSYNTHESIS | AGE OF CULTURE | POPULATION DENSITY | MAXIMUM PHOTOSYNTHESIS |
| *days* | $mm.^3$ | $mm.^3\ O_2$ | *days* | $mm.^3$ | $mm.^3\ O_2$ |
| 4 | 0.27 | 56 | 7 | 0.068 | 34 |
| 5 | 0.90 | 50 | 14 | 0.23 | 31 |
| 6 | 1.3 | 27 | 23 | 0.65 | 25 |
| 8 | 1.9 | 18 | 30 | 0.95 | 18 |
| 11 | 2.5 | 13 | 37 | 1.3 | 13 |
| 13 | 3.0 | 10 | 44 | 1.6 | 12 |
| 17 | 3.3 | 9.9 | 49 | 1.7 | 11 |

decrease is most marked in sun cells so that old cultures are indistinguishable from old shade cultures. Unless a sun culture is examined while it is still thinly populated (that is, while the average internal illumination is high, the color of the light essentially white, or the supply of nutrients undiminished) the difference between it and a shade culture might escape attention completely. Only when surrounded by these conditions does an actively dividing cell population suddenly build up its collective capacity for carrying on the Blackman reaction.

It might be thought that in a rapidly dividing population, the cells would be smaller than in a more stable population and that consequently, in a unit volume of cell material, the total surface of all the cells would be large. The rate of the Blackman reaction might then turn out to be proportional to this total surface or to the total area of some active surface (such as that of the chloroplasts) inside the cells. The cells of *Chlorella pyrenoidosa* are too small (diameter 3 to 5 μ) to measure accurately but it is easy to count in a haemacytometer the number of cells in a measured volume of culture medium and thence to calculate the number in a cubic millimeter packed by centrifuging. Table V shows such counts for sun and shade cultures at all stages of the growth cycle. Work by YIN in this laboratory has shown in the case of *Chlorella vulgaris* that the number of cells in a unit packed

TABLE V

SIZE OF CHLORELLA CELLS IN CULTURES OF DIFFERENT AGES

| SUN CULTURES | | SHADE CULTURES | |
|---|---|---|---|
| POPULATION DENSITY | NUMBER OF CELLS IN 1 MM.³ PACKED VOLUME | POPULATION DENSITY | NUMBER OF CELLS IN 1 MM.³ PACKED VOLUME |
| *mm.³ cells per ml.* | | *mm.³ cells per ml.* | |
| 0.19 | $21 \times 10^6$ | 0.068 | $23 \times 10^6$ |
| 0.12 | 24 | 0.23 | 27 |
| 0.99 | 19 | 0.65 | 22 |
| 0.98 | 18 | 0.95 | 21 |
| 0.95 | 18 | 1.3 | 17 |
| 1.43 | 15 | 1.6 | 18 |
| 1.23 | 15 | 1.7 | 17 |
| Mean | 18.6 | | 20.7 |

volume is determined only by the size of the cells, and not by differences in the closeness of packing. Hence it can be shown that the total area of the surfaces of all the cells in one cubic millimeter is proportional to the cube root of the number of cells. From table V it is apparent that sun cells are so nearly the same size as shade cells that the difference between the cube roots is negligible. Cells from old cultures are, on the average, larger than those from young ones, but the difference is by no means large enough to account for the difference in the rate of the Blackman reaction on the basis of a difference in surface area.

Not only at saturating light intensities, but also at all lower intensities, the rate of assimilation of sun cells is higher than that of shade cells. Figure 1 shows the relation of photosynthesis to light intensity for samples of each variety of cells. The data for these curves and others appear in table VI. It is clear that while the ratio between the rates of sun and shade plants decreases with decreasing intensity, it never drops below 1.0 even in the range where photosynthesis is proportional to light intensity. As previous investigators, without exception, have reported that at low light intensities, shade plants assimilate faster than sun plants, this result is unexpected.

When an attempt is made to compare rates of photosynthesis of cell suspensions at sub-saturating intensities, it is necessary to take into account the decrease of intensity suffered by light traversing the suspension. The laminae of the suspension nearest the source of illumination shade the laminae behind them. This shading would be represented in figure 1 by moving all the points on a curve to the left by an amount proportional to their distances from the y-axis. If the corrections were very different for

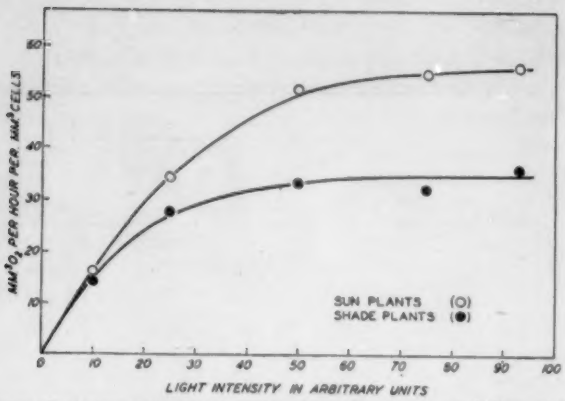



FIG. 1. Rate of photosynthesis of sun and shade plants at different light intensities. The approximate value of the light intensity marked 100 is discussed in the text.

PROTOCOLS FOR FIGURE 1

SUN PLANTS, AUGUST 13, 1936

CULTURE FOUR DAYS OLD. INOCULATED WITH 0.2 MM.³ CELLS. POPULATION DENSITY, 0.27 MM.³ CELLS IN 1 ML. 1.9 MM.³ CELLS IN EACH VESSEL. TEMPERATURE, 25°

| Filter | 10% | 25% | 50% | 75% | 93% |
|---|---|---|---|---|---|
| Reading (60′ light) mm. Brodie | 35.6 | 82.0 | 73.6 | 73.6 | 76.8 |
| Vessel constant | 0.750 | 0.744 | 1.27 | 1.35 | 1.33 |
| $O_2$ evolved (60′ light) | 26.7 | 61.0 | 93.6 | 99.5 | 102.1 |
| $O_2$ consumed (60′ dark) | 4.3 | 4.3 | 4.3 | 4.3 | 4.3 |
| Photosynthesis per vessel | 31.0 | 65.3 | 97.9 | 103.8 | 106.4 |
| mm.³ cells per vessel | 1.9 | 1.9 | 1.9 | 1.9 | 1.9 |
| mm.³ $O_2$ per hour per mm.³ cells | 16.3 | 34.4 | 51.5 | 54.6 | 56.0 |

SHADE PLANTS, AUGUST 27, 1937

CULTURE TWELVE DAYS OLD. INOCULATED WITH 0.6 MM.³ CELLS. POPULATION DENSITY, 0.32 MM.³ CELLS IN 1 ML. 1.1 MM.³ CELLS IN EACH VESSEL. TEMPERATURE, 25°

| Filter | 10% | 25% | 50% | 75% | 93% |
|---|---|---|---|---|---|
| Reading (60′ light) mm. Brodie | 11.3 | 21.9 | 26.3 | 45.8 | 51.9 |
| Vessel constant | 1.27 | 1.35 | 1.33 | 0.750 | 0.744 |
| $O_2$ evolved (60′ light) | 14.4 | 29.6 | 35.0 | 34.4 | 38.6 |
| $O_2$ consumed (60′ dark) | 1.3 | 1.3 | 1.3 | 1.3 | 1.3 |
| Photosynthesis per vessel | 15.7 | 30.9 | 36.3 | 35.7 | 39.9 |
| mm.³ cells per vessel | 1.1 | 1.1 | 1.1 | 1.1 | 1.1 |
| mm.³ $O_2$ per hour per mm.³ cells | 14.3 | 28.1 | 33.0 | 32.4 | 36.3 |

the two curves, the latter might cross. The principal agents of this shading (absorption, and scattering) cannot be eliminated by any practical method or adjusted to equivalence for different suspensions. They can, however, be minimized. The data in table VI were obtained with suspensions containing less than 2 mm.$^3$ of cells in the volume of fluid used in each vessel. Each vessel had a bottom area of about 5 cm.$^2$ The diameters of Chlorella cells range from 3 to 5 μ. A simple calculation shows that if all the cells settle uniformly they would form a layer only one cell deep, which would not entirely cover the bottom of the vessel. In a suspension of such meager density the average illumination within the vessel should be nearly identical with the incident illumination. Hence the correction to be applied to the points in figure 1 would be small and would not affect the relative positions of the two curves.

TABLE VI

THE RATE OF PHOTOSYNTHESIS OF CELLS FROM YOUNG SUN AND SHADE CULTURES AT VARYING LIGHT INTENSITIES. PHOTOSYNTHESIS AND RESPIRATION IN VOLUMES OF OXYGEN PRODUCED BY UNIT VOLUME OF CELLS IN AN HOUR. LIGHT INTENSITIES IN UNITS PROPORTIONAL TO ABSOLUTE INCIDENT INTENSITIES.

| | Light intensity | | | | | |
|---|---|---|---|---|---|---|
| | 10 | 25 | 50 | 75 | 93 | Dark |
| Sun cultures | 16 | 34 | 52 | 55 | 56 | −2.3 |
| | 23 | 44 | 55 | 58 | 61 | −0.6 |
| | 17 | 37 | 55 | 61 | 65 | −1.6* |
| Shade cultures | 14 | 28 | 33 | 32 | 36 | −1.2* |
| | 17 | 30 | 33 | 38 | 39 | −1.5* |
| | 14 | 25 | 26 | 24 | 26 | −1.8 |

* The respiration and respiratory quotient were determined in a separate experiment.

Another possible source of error is the correction for respiration. The rate of respiration, however, in the present experiments was, in every case, small compared with the rate of photosynthesis at even the lowest light intensity used. In those cases marked with an asterisk in table VI the rates of respiration and the respiratory quotient were separately determined with great care. They, as well as all other respiration rates measured in this investigation, were of the same order of magnitude and of an order lower than the rate of photosynthesis. The modifications necessary to change the relative positions of any parts of the curves in figure 1 are larger than any possible errors that could have occurred.

There seems to be no question that sun cells of Chlorella assimilate faster than shade cells even at low light intensities. Before concluding that there is a fundamental difference between Chlorella and the sun and shade plants

investigated by earlier workers, we must examine their experiments in detail. The statements of LUBIMENKO, BOYSEN-JENSEN, and LUNDEGÅRDH are based on readings obtained in the light, not corrected for respiration. They found, however, in every case, that sun plants have a higher rate of respiration than shade plants. The magnitude of the difference is such that, if the readings at low light intensities are corrected by these amounts, the sun plants at every light intensity have a higher true rate of photosynthesis than the shade plants. The conclusion of LUBIMENKO, (**13**), and others, that shade plants are "adapted" to existence in shady places is not affected by this fact, but in terms of the present argument their sun plants assimilated faster than their shade plants at all light intensities.

Shade cells of Chlorella show no inhibition of photosynthesis by light of high intensity. In table VI appear the figures for cells which were saturated by light intensity 25, and showed undiminished photosynthesis under four times as much illumination. Other cases more marked than this were observed. While in very intense light, the shade variety of Chlorella might suffer an inhibition of photosynthesis, no inhibition is apparent at intensities like that of direct sunlight, or at intensities ten to twenty times that in which the cells have grown. There is no sharply optimal intensity for the assimilation of the shade cells as found by MONTFORT and NEYDEL (**16**) for shade varieties of ferns.

The rate of photosynthesis of sun and shade varieties of Chlorella at different concentrations of carbon dioxide was measured in mixtures of tenth molar potassium carbonate and potassium bicarbonate as described by EMERSON and ARNOLD (**6**). The partial pressures of carbon dioxide in equilibrium with these solutions have been calculated from figures given by WALKER, BRAY and JOHNSTON (**18**). The interpretation of results obtained

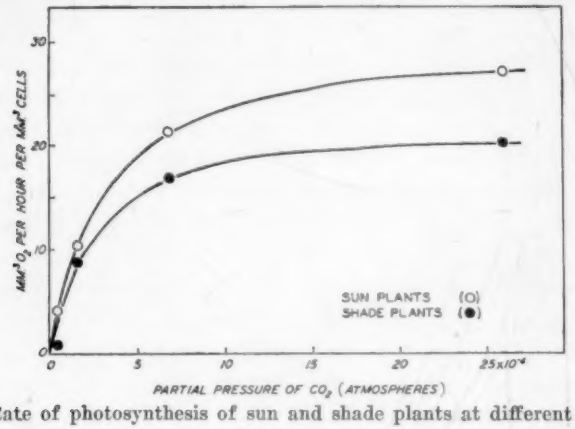



FIG. 2. Rate of photosynthesis of sun and shade plants at different carbon dioxide tensions.

by the use of carbonate-bicarbonate mixtures is at present open to doubt (7). In the absence of a fully accepted method of measuring photosynthesis at low carbon dioxide concentrations, however, data obtained by the present method are at least comparable with much published data obtained by the same method.

The curves relating photosynthesis of sun and shade plants to carbon dioxide concentration appear to be of the same shape (fig. 2). They could be made to coincide by multiplying all the points of either curve by a factor. This factor (1.3 or its reciprocal) is the same as that which relates the rates of the Blackman reaction for the two varieties of plants. Whatever process (or complex of processes) gives the rates measured here, the effect of the sun or shade environment on it is the same as that on the velocity of the Blackman reaction. The rate of carbon dioxide utilization at all partial pressures is independent of the chlorophyll concentration.

The effect of cyanide on the assimilation of sun and shade plants is shown in table VII. The velocity of the Blackman reaction in the sun

TABLE VII

THE EFFECT OF HCN ON PHOTOSYNTHESIS OF CELLS FROM YOUNG SUN AND SHADE CULTURES

| | CONCENTRATION OF HCN | RATE OF PHOTOSYNTHESIS* | INHIBITION |
|---|---|---|---|
| | *molar* | *mm.³ $O_2$* | % |
| Sun | 0 | 42 | 0.0 |
| | $1 \times 10^{-5}$ | 39 | 5.3 |
| | 5 " | 30 | 29 |
| | 10 " | 19 | 54 |
| | 50 " | 6.7 | 83 |
| Shade | 0 | 21 | 0.0 |
| | $1 \times 10^{-5}$ | 20 | 6.7 |
| | 5 " | 16 | 27 |
| | 10 " | 10 | 51 |
| | 50 " | 4.3 | 80 |

* Mm.³ oxygen per hour per mm.³ cells.

plants in this case is twice that in the shade plants, as shown by the values for photosynthesis without cyanide, but the percentage inhibition by any given concentration of cyanide is the same in both varieties of cells.

The photosynthetic quotient, that is, the ratio of carbon dioxide consumption to oxygen production in the light, is the same in sun and shade plants. Within the limits of experimental error it is equal to $-1.00$. The respiratory quotient, the ratio of carbon dioxide to oxygen in the dark, is numerically larger. Table VIII shows that this is the case especially in shade plants. As the calculation of rates of photosynthesis is generally

## TABLE VIII

THE RESPIRATION OF CELLS FROM YOUNG SUN AND SHADE CULTURES

| | RESPIRATORY QUOTIENT | RATE OF RESPIRATION* |
|---|---|---|
| Sun | −1.03 | 3.4 |
| | −1.17 | 1.8 |
| | −1.02 | 1.6 |
| Shade | −1.51 | 0.9 |
| | −1.30 | 1.2 |
| | −1.85 | 1.5 |

* Mm.$^3$ $O_2$ consumed by 1 mm.$^3$ cells in one hour.

based on the assumption that the respiratory quotient is −1.00, this fact would introduce an error, but the rate of respiration is so small compared with that of photosynthesis that the error is negligible. In the only case in which it might be important, the rate of oxygen consumption in the dark was determined directly (table VI). As shown in table VIII, the absolute rate of respiration of sun plants is somewhat higher than that of shade plants.

The actual rates of growth of sun and shade cultures are of some interest. Curves representing these rates are shown in figure 3. The shade

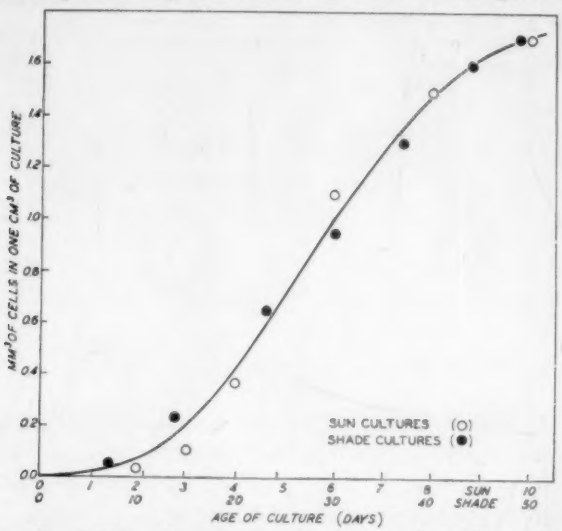



FIG. 3. Growth of Chlorella cultures under sun and shade conditions. The time coordinate for shade cultures is five times as long as that for sun cultures.

cultures in this experiment were grown at a higher light intensity than those reported elsewhere in this paper, namely at 11 cm. from a 40-watt bulb, so that the ratio of the illumination in the sun bath to that in the

shade bath was somewhat higher than 4 to 1. While the ordinates for both curves are the same in figure 3, the abscissae of the shade curve are five times as long as those of the sun curve. By the inclusion of this factor, the curves are superposed except near the origin, which means that the sun cultures grew five times as fast as the shade cultures. This ratio is very near the ratio between the illuminations in the two baths. Unless it is assumed gratuitously that one kind of cells contains more water than the other, this is a measure of the relative rates of photosynthesis. Hence the cells in the cultures assimilate at rates which fall on the rate-intensity curves of figure 1 in the range where assimilation is proportional to light intensity, and the light intensity at which they exhibit maximum photosynthesis is considerably higher than that in which they have been cultivated. The effect of both sun and shade environments is to produce cells whose maximum capacity for assimilation is never realized in their environments.

## Discussion

These experiments show that differences in illumination are sufficient to cause the differences between sun and shade plants observed by earlier workers as noted in the introduction above. When other factors are maintained uniform, plants grown in bright light have a higher rate of photosynthesis and lower chlorophyll content than plants grown in the shade.

Shade plants may have such a low rate of respiration that their excess of photosynthesis over respiration at low light intensities is higher than that of sun plants. In the present experiments shade cultures of Chlorella do not show this "adaptation" to a shade existence but there is a tendency in that direction.

The light optimum for shade plants observed by JOHANNSON (**12**) and by MONTFORT (**15**), does not appear in the present experiments and in those of BOYSEN-JENSEN, LUNDEGÅRDH, and others (**2, 14**). Moreover, optima have been reported for plants which are distinctly sun varieties, namely marine diatoms; thus if optima can be considered as occurring at all, they are not characteristic of shade plants alone. Recently reported experiments by F. GESSNER (**10**) give sound reasons for believing that light optima are artifacts arising from experiments in sealed containers in which insufficient care is taken to avoid stagnation, carbon dioxide deficiency, and consequent damage to the photosynthetic mechanism.

The present experiments give no support to the idea that the lower chlorophyll content of sun plants is to be attributed to the existence of a light sensitive process in which chlorophyll is decomposed. From the rates of growth of sun and shade cultures, and the chlorophyll content of the cells, it follows that during any given period, a unit volume of sun cells produced about two and a half times as much chlorophyll as the same vol-

ume of shade cells. Not all of the implications of this situation are simple but at least it affords no reason for believing that there occurred a continuous decomposition of chlorophyll under the influence of light, which proceeded more rapidly in the sun cultures than in the shade cultures. These experiments however, do not exclude the possibility that such a process occurs.

The present experiments have some bearing on ecological problems. The blooming of marine diatoms and fresh-water algae occurs when the supply of mineral nutrients is large and in a season of intense or prolonged insolation. Under such conditions photosynthesis and, consequently, growth and cell division are rapid. In addition, it appears likely that under these favorable conditions there will be produced plants especially capable of a high rate of photosynthesis. That is, the population of shade plants (or low-nutrient plants) will produce a population of sun plants whose individual capacity for photosynthesis is considerably higher than that of the original population. When the period of favorable conditions comes to an end through either depletion of nutrients or decrease of insolation, the plants will change into the low-nutrient or shade form and their lowered capacity for photosynthesis will compound the effect of the unfavorable conditions. Such a compounding of effects may go far to explain the sudden burst and sudden cessation of growth characteristic of blooms.

That some chlorotic plants reach maturity while other individuals of the same species die early (although by the action of genetic factors, they contain much more chlorophyll (**4**)) may, in some cases, be attributed to processes discussed in this paper. Some individuals genetically poor in chlorophyll may have highly efficient photosynthesis and an unimpaired capacity for the Blackman reaction. At both high and low light intensities they could assimilate as rapidly as individuals with much more chlorophyll. The rate of the Blackman reaction is easily determined and has been measured successfully in a great variety of plants. A study of the inheritance of this factor might yield important information.

## Summary

*Chlorella pyrenoidosa* cultivated under intense illumination has a low chlorophyll content, and a high capacity for photosynthesis. Cultivated under moderately low illumination it has a high chlorophyll content, and a low capacity for the Blackman reaction. The proportional effects of carbon dioxide concentration and cyanide are the same in both varieties of plants. The photosynthetic quotient in both sun and shade plants is equal to – 1.00. The respiratory quotient is absolutely larger especially in shade cells. The average cell size of sun and shade plants is very nearly the same. The rate of growth of the cultures is approximately

proportional to the intensity of illumination. As the population density of the culture increases, the cells undergo internal changes. The average cell size increases; the chlorophyll content (in sun cells) increases; the capacity for the Blackman reaction decreases.

The author expresses his indebtedness to Mr. Robert Emerson and Mr. Lowell F. Green for numerous suggestions and constant assistance in carrying out these experiments.

Scripps Institution of Oceanography
La Jolla, California

## LITERATURE CITED

1. Arnold, William, and Kohn, Henry I. The chlorophyll unit in photosynthesis. Jour. Gen. Physiol. **18**: 109–112. 1934.
2. Boysen Jensen, P. Studies on the production of matter in light- and shadow-plants. Bot. Tidsskr. (Copenhagen) **36**: 219–259. 1918.
3. ————, and Müller, D. Über die Kohlensäureassimilation bei Marchantia und Peltigera. Jahrb. wiss. Bot. **70**: 503–511. 1929.
4. Demerec, M. Behavior of chlorophyll in inheritance. Cold Spring Harbor Symp. Quant. Biol. **3**: 80–86. 1935.
5. Emerson, R. The relation between maximum rate of photosynthesis and concentration of chlorophyll. Jour. Gen. Physiol. **12**: 609–622. 1929.
6. ————, and Arnold, William. The photochemical reaction in photosynthesis. Jour. Gen. Physiol. **16**: 191–205. 1932.
7. ————, and Green, Lowell. Effect of hydrogen-ion concentration on Chlorella photosynthesis. Plant Physiol. **13**: 157–168. 1938.
8. Fleischer, Walter E. The relation between chlorophyll content and rate of photosynthesis. Jour. Gen Physiol. **18**: 573–597. 1935.
9. Gaffron, H. Methoden zur Untersuchung der Kohlensäureassimilation. Abderhalden's Handbuch der biologischen Arbeitsmethoden **11**: 101–160. 1929.
10. Gessner, F. Die Beziehung zwischen Lichtintensitat und Assimilation bei submersen Wasserpflanzen. Jahrb. wiss. Bot. **86**: 491–526. 1938.
11. Harder, R. Bemerkungen über die Variationsbreite des Kompensationspunktes beim Gaswechsel der Pflanzen. Ber. d. bot. Ges. **41**: 194–198. 1923.
12. Johannson, N. Zur Kenntnis der Kohlensäureassimilation einiger Farne. Svensk Bot. Tidskr. **17**: 215–223. 1923.
13. Lubimenko, M. W. Sur la sensibilité de l'appareil chlorophyllien des

plantes ombrophiles et ombrophobes. Rev. Gén. Bot. **17**: 381–415. 1905.

14. Lundegårdh, H. Ecological studies in the assimilation of certain forest-plants and shore-plants. Svensk Bot. Tidskr. **15**: 46–95. 1921.
15. Montfort, Camill. Die funktionelle Einstellung verschieden gefärbten Meeresalgen auf die Lichtintensität. Jahrb. wiss. Bot. **71**: 106–148. 1929.
16. ————————, and Neydel, Karl. Zur Beurteilung der "Inaktivierung" und des "Zeitfaktors" bei der Assimilation Stomata-freier Schatten-Farne. Jahrb. wiss. Bot. **68**: 801–843. 1928.
17. Stålfelt, M. G. Zur Kenntnis der Kohlehydratproduktion von Sonnen- und Schattenblättern. Medd. från Statens Skogsforsoksanst. **18**: 221–280. 1921.
18. Walker, A. C., Bray, U. B., and Johnston, J. Equilibrium in solutions of alkali carbonates. Jour. Amer. Chem. Soc. **49**: 1235–1256. 1927.
19. Warburg, O. Über die Katalytischen Wirkungen der Lebendigen Substanz. Berlin. 1928.
20. Willstätter, R., and Stoll, A. Untersuchungen über die Assimilation der Kohlensäure. Berlin. 1918.

# AUXIN IN MARINE ALGAE

J. VAN OVERBEEK

(WITH ONE FIGURE)

## Introduction

Auxin has been studied almost exclusively in the higher land plants. Therefore, it seemed of interest to obtain some information regarding auxin in very different groups such as the lower water plants. For this purpose Macrocystis was selected. *Macrocystis pyrifera* (L.) Ag. is a large brown alga growing on rocks, usually in 10 to 30 meters of water. A description can be found in SETCHELL and GARDNER (**11**), or OLTMANNS (**10**). The stipe ("stem") becomes 30 to 50 meters long, bearing at its tip a terminal, bladderless, falcate blade and lower down the young blades and along the greater part of its length at regular intervals the mature, lateral blades, each with a pyriform bladder at its base. The mature blades are from 3 to 5 dm. long and 5 to 9 cm. wide. The upper 2 to 10 meters of the thallus usually floats on the surface of the water. The alga is perennial, which offers the advantage that material can be collected throughout the year. Since it was not considered practical to investigate the entire plant, only a young apical part about 50 cm. in length was used. Such a part consists of a terminal blade and a stipe to which are attached on the average 15 blades and bladders of an increasing size toward the base. Before the physiological rôle of auxin of a plant can be determined it is desirable to: (a) demonstrate the presence of auxin; (b), to determine its distribution throughout the plant; and (c) to discover the type of the auxin involved. These are the three points which will be discussed in this paper.

VAN DER WEIJ (**12**) in 1933, for the first time demonstrated the presence of auxin in marine algae. In the cell sap of young *Valonia macrophysa* plants he found between 0.00012 and 0.00021 mg. of auxin per liter, and in the cell walls of the same material about twenty times that amount. DU BUY and OLSEN (**3**) extracted auxin from Fucus. During the summer of 1938 I determined the auxin content of the green alga Bryopsis which had been collected and extracted (**14, 15**) by MR. M. L. DARSIE at Pacific Grove, California. About 80 gamma equivalents of indole acetic acid per kg. fresh weight were found. This is an auxin concentration of the same order as occurs in pea seedlings, but considerably smaller than found in Avena and corn plants (**13, 14, 15**). Another water plant in which such relatively large auxin concentrations were found is Elodea, in which I found auxin concentrations as high as 50 gamma equivalents of indole acetic acid per kg. fresh weight. In Macrocystis, on the other hand, auxin concentrations

of only about one gamma per liter were found, which is close to VAN DER WEIJ's values for Valonia.

## Experimental methods and results

Young undamaged plants free from overgrowths, as far as could be determined macroscopically, were collected in a pail of sea water. The plants were collected by boat at the kelp beds near the breakwaters of Newport Bay, or off the coast. Material that had become detached or was washed ashore was never used. Within one half hour after collection, the material was thoroughly washed with fresh water in order to remove microorganisms that might be attached, and then placed in highly purified ethyl ether. It was left in the ether for 2 to 3 days, and after that time the assay for auxin was made in a previously described manner (**14, 15**). However, one further simplification was introduced (suggested by DR. W. S. STEWART). Instead of evaporating the residue to complete dryness and adding agar to it, the extract was evaporated down to about 2 ml. which were then dropped immediately into the hot agar. In this way the auxin was instantaneously mixed with the agar and the 2-hour period of standing which was previously necessary to insure this mixing was eliminated. Each test included a control curve with known amounts of indole acetic acid which made it possible to bring the threshold value into calculation (**16**).

In order to check whether or not all of the auxin was removed, the extraction method described above was compared with the Soxhlet method, which is one of the most exhaustive methods known. Table I shows that practically the same amount was obtained by both methods. Hence, one can be reasonably sure that the data presented here truly represent the auxin content of the material.

TABLE I

AMOUNTS OF AUXIN* OBTAINED FROM MACROCYSTIS BLADES BY MEANS OF EXTRACTION IN ETHER FOR 2 DAYS AND BY SOXHLET EXTRACTION DURING THE SAME TIME (81101)

| PART OF PLANT | SOXHLET | STANDING |
|---|---|---|
| Terminal blade | 0.61 | 0.73 |
| Young lateral blades | 1.13 | 1.00 |

* Gamma equivalents of indole acetic acid per kilogram fresh weight.

## Distribution

As was stated in the introduction, only the apical 50 cm. of the thallus was investigated. In table II a typical distribution experiment is represented which shows all of the individual factors necessary for the determination of the auxin concentration in the plant. In the first column is indicated the part of the thallus and the number used for extraction. The column marked W indicates the total fresh weight in grams of the parts

## TABLE II

A COMPLETE ANALYSIS OF THE AUXIN DISTRIBUTION IN THE APICAL PART OF A MACROCYSTIS THALLUS. EXPLANATION OF SYMBOLS IN TEXT (81026)

| | W | $V_a$ | C | $(C \times I_{1°} + 0)$ | Gamma / Kg. | Gamma / Part |
|---|---|---|---|---|---|---|
| | *gm.* | *ml.* | *°* | | | |
| 10 terminal blades | 17.5 | 0.5 | 6.5 | 19.0 | 0.54 | $87 \times 10^{-5}$ |
| | | | 5.3 | 16.0 | 0.46 | |
| 10 stipes | 42.0 | 0.3 | 7.2 | 20.0 | 0.14 | $59 \times 10^{-5}$ |
| 100 bladders | 59.0 | 0.5 | 6.8 | 19.5 | 0.16 | $9 \times 10^{-5}$ |
| | | | 7.0 | | | |
| 15 lateral blades | 62.0 | 0.5 | 7.0 | 20.0 | 0.16 | $78 \times 10^{-5}$ |
| | | | 10.5 | 27.5 | 0.22 | |
| Controls | | | | | | |
| 26.5 gamma indole acetic acid per liter gave C = − 10.0° | | | | | | |
| 13.2 gamma indole acetic acid per liter gave C = − 4.1° | | | | | | |
| 0.0 gamma indole acetic acid per liter gave C = + 1.8° | | | | | | |

under investigation. The column $V_a$ indicates the volume of agar in ml. in which the extract was taken up, C gives the degree of curvature in the Avena test. The factor $(C \times I_{1°} + 0)$ translates degrees of curvature into concentration of indole acetic acid. Its value can be directly determined from the concentration curve with known amounts of indole acetic acid. $I_{1°}$ is the indole acetic acid concentration required to give an increase of curvature in the Avena test of 1°. 0 is the threshold value in gammas indole acetic acid per liter. The auxin concentration in gamma equivalents of indole acetic acid per liter (kg. fresh weight) is calculated from:

$$\frac{(C \times I_{1°} + 0) \times V_a}{W}$$

which is given in the sixth column. The last column finally gives the auxin content of an average terminal blade, stipe, bladder, or young lateral blade.

As table II indicates, the auxin concentration is highest in the terminal blade and lowest in the stipe. This relation was generally found, but the terminal blade does not always have a higher auxin concentration than the upper lateral ones, as follows from table I. In table III a summary of all available data on the auxin distribution is given. There appears to be a definite decrease in auxin concentration in the basal direction although this auxin gradient is not as pronounced as in seedlings of higher plants (**14, 15**). The low auxin concentration in the rapidly growing stipe may be compared with a similar situation in young corn seedlings (**15**). Here it was found that the upper part of the first internode, which is the fastest

TABLE III

DISTRIBUTION OF AUXIN* IN THE UPPER PART OF THE THALLUS OF MACROCYSTIS

| TERMINAL BLADE | YOUNG BLADES | OLDER BLADES | BLADDERS | STIPE | EXPERIMENT NUMBER | DATE |
|---|---|---|---|---|---|---|
| 1.75 | 1.00 | 0.53 | ..... | ..... | 80926 | (Sept. 26, 1938) |
| 0.84 | 0.19 | ..... | ..... | 0.21 | 81019 | (Oct. 19, 1938) |
| 0.51 | ..... | ..... | ..... | ..... | | |
| 0.54 | 0.16 | ..... | .16 | 0.14 | 81026 | (Oct. 26, 1938) |
| 0.46 | 0.22 | ..... | ..... | ..... | | |
| 0.61 | 1.13 | ..... | ..... | ..... | 81101 | (Nov. 1, 1938) |
| 0.73 | 1.00 | ..... | ..... | ..... | | |

* Gamma equivalents of indole acetic acid per kilogram of fresh weight.

growing region of the entire corn seedling, has the lowest auxin content. It may be that in these cases the rapidly growing tissue consumes large amounts of auxin as discussed later.

### TYPE OF AUXIN

In order to determine the nature of the auxin present in Macrocystis three types of investigation are open.

(a). The most satisfactory is to obtain it in crystalline form and determine its structure in a manner similar to that followed by KÖGL and HAAGEN-SMIT for auxin-a,-b, and indole acetic acid. A glance at tables I, II, and III shows that only about one-half part of auxin per billion ($10^9$) parts of Macrocystis is present. Hence it would require tons of material to isolate a fraction of a gram of auxin, which makes direct determinations impracticable in this case.

(b) KÖGL, HAAGEN-SMIT, and ERXLEBEN (7) have described a convenient differential acid-alkali destruction test which makes it possible to distinguish between indole acetic acid which is destroyed by acid but stable in alkali, auxin-a which is stable in acid but destroyed by alkali, and auxin-b which is unstable both in acid and alkali. When this method was tried on extracts of Macrocystis blades, invariably the auxin present in it was destroyed by acid, but not by alkali, indicating that indole acetic acid was present. Table IV gives the results of a few experiments of this type. It is noteworthy that in several instances refluxing with sodium hydroxide increased the auxin activity of the extract over that which had been boiled with distilled water. It may be that by boiling with dilute NaOH active auxin is liberated from a precursor, but it is also possible that some inhibitory substance is inactivated. Since HAAGEN-SMIT (private communication) does not consider the differential destruction test reliable for impure ex-

TABLE IV

AUXIN CONTENT* OF MACROCYSTIS EXTRACTS

| REFLUXING WITH 5 PER CENT. HCl | REFLUXING WITH 1 N NaOH | REFLUXING WITH DISTILLED WATER | CONTROL NOT REFLUXED | TIME | EXPERIMENT NUMBER |
|---|---|---|---|---|---|
| *deg.* | *deg.* | *deg.* | *deg.* | *min.* | |
| +0.1 | 11.3 | 5.5 | ..... | 45 | 81025 |
| +1.5 | 10.0 | 6.3 | ..... | ..... | |
| 2.3 | 14.1 | 12.3 | 12.8 | 30 | 81019 |
| 2.9 | 13.5 | 10.7 | 11.6 | ..... | |

* Degrees of curvature in the Avena test.

tracts, the third method which is available for obtaining information about the nature of the auxin was also tried.

(c) This method is based upon the determination of the diffusion coefficient and was first successfully applied by WENT (**17**), who four years before the auxin-a was chemically isolated, had determined its molecular weight. The experiment was carried out as follows: the auxin present in an extract of Macrocystis was taken up in a small agar plate ($10.8 \times 8.1 \times 0.8$ mm.). This plate was then carefully placed on top of a stack of three similar plates containing no auxin. After 40 minutes the 4 plates were separated and the auxin content in each of them determined by the Avena test method. From the relative distribution of the *actual amount* of auxin (rather than Avena curvature) in each of the four plates the diffusion coefficient can be found from diffusion tables after SHEFFER and KAWALKI (**1**). Once the diffusion coefficient (D) is known, the molecular weight can be calculated from $\sqrt{M} = \frac{7 \times 1.07}{D_{22^\circ}}$. Since it is extremely important to know the relative distribution of the actual auxin content in the four agar plates, one is not justified in directly using the degrees of curvature obtained in the Avena test for the determination of the relative auxin distribution in the tables, unless one is sure that there is a direct proportionality between the Avena curvature and the actual auxin concentration in the agar plates. Such a direct relationship does not exist in a great many cases. The curve did not start at the origin, but somewhere on the concentration axis, indicating that there was a threshold value. In order to avoid this error a diffusion test was run with indole acetic acid ($M = 175$) parallel to the diffusion test with the unknown auxin. In this control run the expected relative distribution of the indole acetic acid in the 4 agar plates may be found from the tables. The Avena curvature for each of these 4 plates is found by means of the Avena test. When these Avena curvatures are

plotted against the concentration of auxin obtained from the tables an accurate concentration curve for the conditions of the experiment is obtained. This curve subsequently was used to translate the Avena curvature of the diffusion test with the unknown auxin into relative amounts of auxin. When this was done diffusion coefficients and molecular weights for the auxin in Macrocystis were found which were close to those of indole acetic acid (table V). The difference between the molecular weight found for

TABLE V

DIFFUSION AND MOLECULAR WEIGHTS FOR AUXIN IN MACROCYSTIS. EXPLANATION IN TEXT

| | $D_{22°}$ | MOLECULAR WEIGHT | EXPERIMENT NUMBER |
|---|---|---|---|
| Auxin-a | 0.414 | 328 | ..... |
| Auxin-b | 0.426 | 310 | ..... |
| Indole acetic acid | 0.567 | 175 | ..... |
| Auxin from Macrocystis | 0.612 | 149 | 81117 |
| | 0.599 | 156 | 81207 |

Macrocystis-auxin and that of indole acetic acid is probably not significant. It is clearly shown, however, that the auxin of Macrocystis cannot be auxin-a or -b.

In determining the molecular weight by means of the diffusion test it was found inadvisable to work with a donor plate which contained an excessive amount of impurities. Such a block gives too low values in the Avena test. It was found that when such an impure block was placed for one hour on top of a block of plain agar, the latter gave a higher curvative than the former. Auxin had diffused into this plate of agar, but the majority of impurities remained behind in the original block. In the experiments mentioned in table V, use was made of this simple method of purification.

## Discussion

It is generally assumed that higher plants contain auxin-a and -b and that lower ones contain indole acetic acid. This generalization is based upon the actual isolation of auxin-a and -b from corn oil and malt (**6**), and by a number of molecular weight determinations for higher plants (**4, 7**) all of which showed that auxin of a molecular weight like that of auxin-a or -b is present in these plants. The lower plants so far investigated have been fungi and bacteria. Indole acetic acid was actually isolated from yeast and Rhizopus (**2, 5, 7**), and more indirect evidence for the presence of indole acetic acid was obtained in Aspergillus and *Bacterium coli* (**9, 10**). The lower plants so far investigated were saprophytes in contrast to the

autotrophic higher plants. It is interesting to see that in the case of Macrocystis, an autotrophic lower plant, indole acetic acid or a substance closely related to it is present.

Although auxin has now been demonstrated in at least 3 marine algae, its mere presence does not make it a growth hormone for these organisms. Further researches have been planned in order to elucidate this particular point. There is evidence, however, that auxin is a growth hormone for Macrocystis: (1) the higher auxin concentration in the blades of the apical growing part of the thallus than in the older mature part. (2) The similarity in the relation between growth and auxin distribution in corn seedlings and in the stipe of Macrocystis. In an earlier paper (**15**) it was shown that in young corn seedlings the region of maximal rate of elongation is located in the upper part of the first internode. It was also shown that out of this particular region the smallest amount of auxin of the entire plant could be extracted. Strange though this may seem at first sight the following consideration will show that it is to be expected. If one assumes that auxin is used up during the process of elongation one can expect that the faster a particular region elongates the more auxin it uses. In the upper region of the corn coleoptile the growth rate is relatively small and is not limited by auxin but by other factors. In the lower part of the coleoptile and the upper part of the first internode elongation is limited by auxin. This has been shown by decapitation experiments (**15**). In this upper region of the first internode the other factors necessary for elongation are present in excess, and auxin will cause it to elongate as soon as it arrives at the proper spot. It is thereby rendered inactive. When active auxin is extracted it will be auxin which was present in the plant under the following conditions: (a) on its way to those regions where it will cause elongation; (b) on its way to more basal regions; and (c) as bound auxin. For a discussion of bound and free-moving auxin see (**18**) and a paper by the writer on "Auxin in roots."[1]

It will be clear from the above consideration that in the upper part of the coleoptile, where the auxin is produced and relatively little is used for elongation, relatively large amounts are found upon extraction. On the other hand, the more basal parts of the corn seedling (the upper part of the first internode) will have a relatively low auxin concentration because: (1) they receive only auxin which has been left over by the more apical regions; and (2) since the other growth factors are present in excess, practically all the auxin will be used for elongation. If a similar relation between elongation and auxin content exists in the stipe of Macrocystis, the relatively low auxin content there may be regarded as an indication that its elongation is auxin controlled.

[1] Bot. Gaz. **101**: 450–456. 1939.

## Summary

In the brown alga Macrocystis auxin is present in a concentration of approximately 0.5 gamma equivalents of indole acetic acid per kilogram fresh weight. In the green alga Bryopsis up to 80 gammas per kilogram, and in Elodea 50 gammas per kilogram fresh weight were found. These are auxin concentrations of the same order of magnitude as are present in higher plants such as corn (on the average 0.5 gamma equivalents of indole acetic acid per kg.) and pea seedlings (on the average 50 gammas per keg.). The auxin distribution in the upper 50 cm. of the thallus was investigated. The young blades had the highest auxin concentration and the stipe (stem) the lowest (fig. 1). It has been shown that the auxin of Macrocystis is indole acetic acid, or a substance closely related to it, rather than auxin-a or -b.

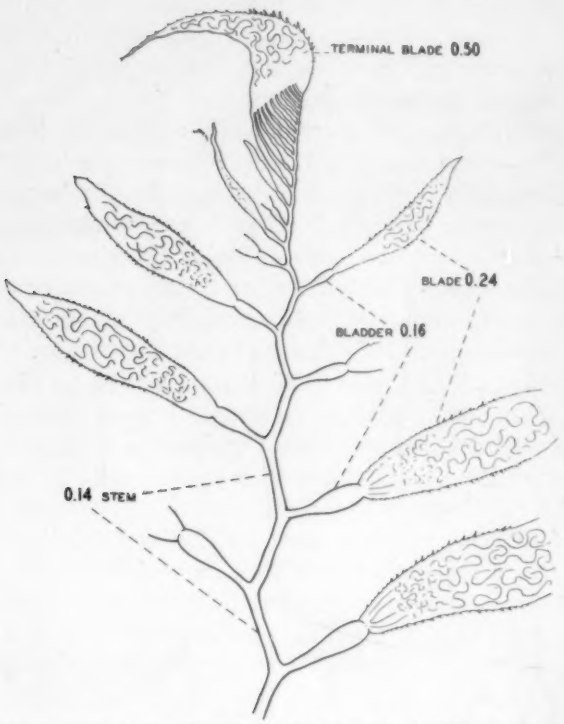




FIG. 1. Apical part of the thallus of *Macrocystis pyrifera* with figures indicating the distribution of auxin in gamma equivalents of indole acetic acid per kilogram fresh weight.

THE CALIFORNIA INSTITUTE OF TECHNOLOGY
PASADENA, CALIFORNIA

## LITERATURE CITED


1. ARVELT, K. Handbuch der physikalisch-chemischen Technik. F. Erdke, Stuttgart. 1923.
2. DOLK, H. E., and THIMANN, K. V. Studies on the growth hormone of plants. I. Proc. Nat. Acad. Sci. **18**: 30–46. 1932.
3. DU BUY, H. G., and OLSON, R. A. The presence of growth regulators during the early development of Fucus. Amer. Jour. Bot. **24**: 609–611. 1937.
4. HEYN, A. N. J. The chemical nature of some growth hormones as determined by the diffusion method. Proc. Kon. Akad. Wetensch. Amsterdam **38**: 1074–1081. 1935.
5. KÖGL, F. Wirkstoffprinzip und Pflanzenwachstum. Naturwiss. **25**: 465–470. 1937.
6. ————, ERXLEBEN, H., and HAAGEN-SMIT, A. J. Über die Isolierung der Auxine a und b aus pflanzlichen Materialen. Zeitschr. physiol. Chem. **225**: 215–229. 1934.
7. ————, HAAGEN-SMIT, A. J., and ERXLEBEN, H. Über ein neues Auxin (Hetero-auxin) aus Harn. Zeitschr. physiol. Chem. **228**: 90–112. 1934.
8. ————, and KOSTERMANS, D. G. F. R. Hetero-auxin als Stoffwechselprodukt niederer pflanzlichen Organismen. Isolierung aus Hefe. Zeitschr. physiol. Chem. **228**: 113–121. 1934.
9. KOSTERMAN, D. G. F. R. Over hetero-auxine Thesis, Utrecht. 1935.
10. OLTMANNS, FR. Morphologie und Biologie der Algen II. Fischer, Jena. 1922.
11. SETCHELL, W. A., and GARDNER, N. L. The marine algae of the Pacific Coast of North America. Univ. California Public. 8 (3). 1925.
12. VAN DER WEIJ, H. G. On the occurrence of growth substances in marine algae. Proc. Kon. Akad. Wetensch. Amsterdam. **36**: 759–760. 1933.
13. VAN OVERBEEK, J. "Laziness" in maize due to abnormal distribution of growth hormone. Jour. Hered. **29**: 339–341. 1938.
14. ————. A simplified method for auxin extraction. Proc. Nat. Acad. Sci. **24**: 42–46. 1938.
15. ————. Auxin distribution in seedlings and its bearing on the problem of bud inhibition. Bot. Gaz. **100**: 133–166. 1938.
16. ————, and BONNER, J. Auxin in isolated roots growing *in vitro*. Proc. Nat. Acad. Sci. **24**: 260–264. 1938.
17. WENT, F. W. Wuchsstoff und Wachstum. Rec. trav. bot. néerl. **25**: 1–116. 1929.
18. ————, and THIMANN, K. V. Phytohormones. Macmillan, New York. 1937.

# SPIRAL SYSTEMS IN THE ORGANIZATION OF LIVING MATERIAL

W. D. FRANCIS

(WITH FIVE FIGURES)

## Introduction

Since the invention of the microscope the problem of the structure of protoplasm has maintained a very prominent place in the study of life. Many investigators have hoped that the discovery of a mechanical structure in living material would yield some explanation of the remarkable functions connected with life. The colloidal concept of living material contributed much to the understanding of life processes, particularly in connection with surface reactions. On the other hand, the colloidal concept in so far as it is contrasted in a structural sense with the crystalloidal concept lent little or no support to the microscopist in his search for organized structure in living material. The recognition that the intricate structures and coordinated functions of organisms are compatible only with an orderly arrangement of living material brought the mind back to some such structure as crystals exhibit (**5, 14**). Thus arose the present conception of protoplasmic constitution as being largely liquid crystalline.

Plant and animal protoplasm are generally assumed to be fundamentally similar. One basis of this assumption is that both kinds of protoplasm exhibit the general phenomena of life. Another basis for the assumption is the difficulty of differentiating whole groups of lower organisms as distinctively plant or animal in character. From these considerations one could expect that a definite structure which is found to be fundamental in the protoplasm of one species may be common to the protoplasm of all species.

## Discussion

### THE SPIRAL STRUCTURE OF CHROMOSOMES AND CYTOPLASM

The spiral structure of chromosomes and their finer deeply staining filaments or chromonemata has been established by recent work (**7**).

It has been remarked by SCARTH (**12**) that there is no occasion to suppose that the cell is ever without a skeleton of organized and probably orientated elements. The spiral structure of cytoplasm was observed by the writer (**6**) in a photomicrograph by SEIFRIZ (**13**) of the quiescent protoplasm of a slime mold.

One very readily and unconsciously assumes that microscopic vision is similar to ordinary vision with the unaided eye. As an example we may take the stamen hairs of *Rhoeo discolor*, which are sufficiently fine and transparent to be examined with the best immersion lenses. When the hairs are

mounted in water and examined under the microscope, the striated cellulose wall on the upper side comes into view and can be accurately resolved. Next the protoplasm within the hair cells can be focussed and seen clearly at any desired level. Finally, the cellulose wall on the lower side can be brought into view and its fine structure studied. It is to be remarked, however, that as the focus is altered either downwards or upwards the previous upper or lower field of view disappears. Thus microscopic vision at any one time is restricted to one plane which is roughly at right angles to the optical axis of the microscope. The higher the numerical aperture of the lens, and consequently the higher its resolving power, the more restricted is its field towards the plane of its sharpest focus. This peculiarity of microscopic vision accounts for the circumstance that insufficient attention has been given to structures which are beyond the field of view, but which are parts of the structures within the field of view. Gross structures such as cell walls are generally readily correlated with their parts, which are outside the field of view, by focussing. Very fine structures such as disperse aggregations and spirals, however, which are partly inside and partly outside the field of view, are often difficult to perceive and interpret. It is particularly pertinent to observe that the filament which composes the spiral, or helix, which we are discussing, never lies in one plane.

The granular appearance of protoplasm which is so often presented to the observer by the microscope is evidently due in great measure to optical sections through a spirally arranged disperse phase. The apparent granules are often round or slightly elongated images provided by spirally wound filaments as they are traversed crosswise or obliquely by the optical section provided by the microscope. Figure 1 is a diagram of a longitudinal section of a spiral. The diagram shows that a spiral in the position indicated is represented by two rows of dots, the dots of one row being placed alternately to the dots of the other row.

FIG. 1. Diagram of longitudinal section through a spiral. The spirally coiled filament appears as two rows of dots. The dots of one row alternate with the dots of the other row. When reversals in direction of the spirals occur, as in the spirals of chromosomes and cytoplasm, the pattern of dots is more irregular.

The limitation of microscopic resolution to sharply defined optical sections is strikingly revealed by the photomicrographs in figures 2 and 3. In parts of both of these photomicrographs fine protoplasmic connections are shown traversing the cellulose walls.

The advantage of photomicrography is that details which are invisible to the eye in the ordinary routine of microscopical observation are often shown up in a photomicrograph. Two examples will be given. Because of their recognized advantages, apochromatic lenses were used. On one occasion the writer photographed a precipitate of ferrous hydroxide which was mounted

on a slide in Canada balsam. The resulting plates showed long straight lines consisting of crystal edges and elongated crystals. These straight lines could not be found in the Canada-balsam preparation by visual observation. Later similar straight lines were detected by visual observation in preparations of the same material mounted in water. Evidently differences in the refractive properties of the mounted material and the mounting medium, which were insufficient to be detected by the eye, were registered on the photographic plate.

On another occasion a mounted preparation of iron rust was photographed under the microscope. The resulting picture clearly showed spiral structure in the rust. The writer had previously examined iron rust on numerous occasions without having observed its spiral structure. After it could be revealed by photographs, the spiral structure was detected in iron rust by ordinary visual methods with the microscope.

SEIFRIZ's photomicrograph (**13**) showing spiral structure in protoplasm led the writer to make photomicrographs of living protoplasm. The epidermis of the bulb scale of the onion (*Allium cepa*), freshly mounted in tap water, was the subject chosen. Figures 2 and 3 are reproductions of some of the photomicrographs. The photomicrographs in this paper are of the "silver skin" type of onion.

The photomicrographs show spiral structure in the protoplasm, in the oil bodies suspended in the protoplasm, and in parts of the cellulose walls. The oil bodies were seen in the protoplasm before the photographs were taken. They are often irregular, angular, or rounded in shape and strongly light-refracting. The cellulose walls in the "silver skin" variety were also strongly light-refracting. This light-refracting property which is rendered by the more pale color differentiates the oil bodies and cell walls from the background of the protoplasm in the photomicrographs. Within the oil bodies as well as in the protoplasm are dark spiral filaments. From visual studies and from photomicrographs in which the material composition of the structures is known one may interpret these dark spirals as protein. For example, the darker portions of the nucleus both in visual observations and in photomicrographs consist of the chromonemata which are composed of protein. KUWADA and NAKAMURA's photomicrograph (**8**) of a dividing nucleus in a living stamen hair of Tradescantia shows the chromonemata as dark twisted filaments. Confirmation of the interpretation of the dark spirals as protein is provided by ANDREWS (**2**), who found that the oil bodies of plants consist of oil and a protein base.

The distribution and character of the oil of the onion and allied species is outlined by MOLISCH (**11**). In common with many other authors he refers to the oil as allyl sulphide. CZAPEK (**3**) remarks that allyl-propyl disulphide appears to be an important constituent of the oil of the onion. From the

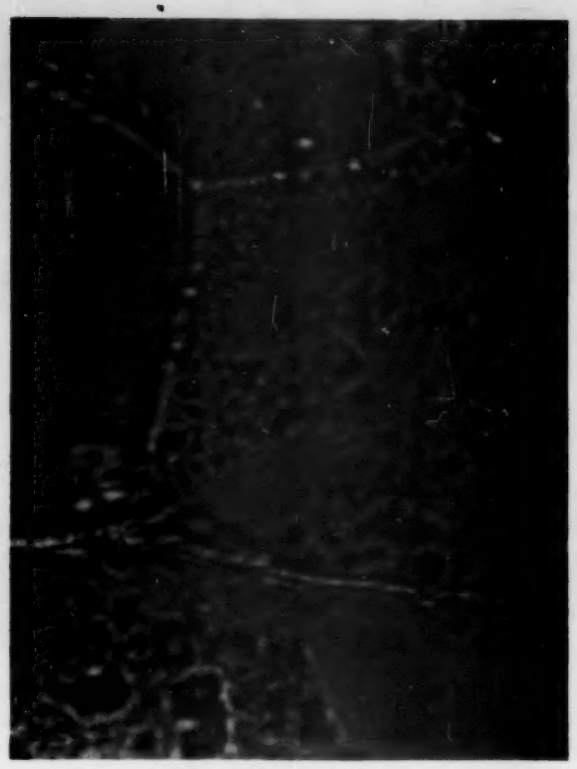


FIG. 2. Unretouched photomicrograph of living protoplasm of epidermis of bulb scale of onion. Apochromat 2 mm. n.a.1.33. × 1250. The pale light-refracting areas in the cells represent onion oil. This is shown as spirals, or sections of spirals, often surrounding dark areas evidently of protein. The spiral character of the dark proteinaceous parts is well shown on the left of the lowermost cell where the spiral protein structures are invested by the pale light-refracting spirally arranged oil. The cell wall in the left upper corner is shown as coiled in two strands consisting of left-hand spirals. The appearance of the cell wall here is suggestive of a cord composed of two strands. Indications of left-hand spiral structure are also shown in the cell wall towards the lower part of the photograph. A few fine protoplasmic connections are shown piercing the cell wall in the upper part of the photograph. The discontinuity of the cell wall on the left is evidently caused by the optical section traversing coarse perforations in the cell wall.

location and description of onion oil as outlined by MOLISCH and others it is evident that it is the strongly light-refracting, spirally arranged material appearing in variously shaped bodies in the photomicrographs presented here (figs. 2, 3).

According to LEPESCHKIN (9), protein and lipoids are the principal constituents of protoplasm, of its dispersion medium, and of its disperse phases. He defines lipoids as subtances which are found in organisms and

which are distinguished by their solubility in ether, benzol, chloroform, oil, and partly in alcohol and their insolubility in water. According to this definition onion oil would be included with lipoids.

In accordance with this broad definition of lipoids the writer's photomicrographs provide a picture of the coarse disperse phases of the principal materials of protoplasm; namely, the protein and the lipoid. In LEPESCH-

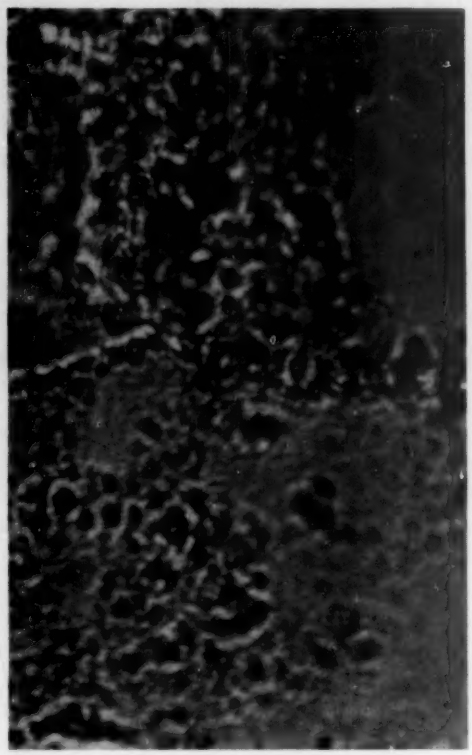


FIG. 3. Unretouched photomicrograph of living protoplasm of epidermis of bulb scale of onion. Apochromat 2 mm. n.a.1.33. Enlarged to ×1143 from original photograph, ×750. The coarse and fine dark twisted spirals in different parts of the photograph are evidently proteinaceous. The pale, light-refracting portions investing the dark spirals represent onion oil; their spiral formation is more evident in the upper part of the photograph. In the upper left portion there are systems of fine and coarse spirals connected by fine anastomoses. The upper part of the photograph is strongly suggestive of protoplasmic organization on the basis of interconnecting spiral systems of descending orders of size. Fine protoplasmic connections are shown perforating the cell wall which passes across the middle of the photograph. It is to be observed that the protoplasmic connections shown in this figure and in figure 2 are dark colored, indicating their proteinaceous character.

KIN's photomicrographs (9, fig. 16, 18) of living protoplasm, alternately dark and light areas are shown. These may represent areas in which protein and lipoids respectively are dominant.

In parts of the writer's photomicrographs the coarse disperse phases of the protoplasm are shown as a lining in contact with the cell wall. In these places the outer layer of the protoplasm would present a surface composed of alternating spiral coils of protein and lipoid. See for example the lower side of the cell wall in figure 3. This photomicrographic feature of the outer layer of protoplasm is significant in connection with NATHANSOHN's conception of the "plasma membrane" as possessing a mosaic structure consisting of proteins alternating with lipoids (9, p. 147.)

In the upper left-hand side of figure 3 there are indications of an orderly arrangement of the spiral systems. Several large spirals are shown passing downwards from near the top of the photograph. One of these spiral systems, the one on the left, is diagrammatically represented in figure 4. It is to be observed that the larger spiral consists of a filament which is wound in a spiral of a lower and much finer order than that of the large coils. Further, it is observable in the upper left-hand

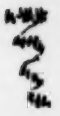


FIG. 4. Diagram of a spiral system shown on upper left of figure 3. The larger spiral coils are composed of a filament which is wound into a spiral of a lower order of size.

side of figure 3 that the large spiral systems, such as those shown diagrammatically in figure 4, are connected with adjacent spiral systems by fine connecting strands (fig. 5). These fine connecting strands may be parts of spirals which are intertwined with the spirals which they appear to connect. The connecting strands are apparently similar to the

FIG. 5. Diagram of portions of two spiral systems, upper and lower, from upper left side of figure 3. The two spiral systems are connected by two anastomoses.

anastomoses of the chromatinic reticulum of nuclei.

The writer has frequently noticed while examining living protoplasm that spirals appear to be visible with lenses of ascending magnifications such as with 16, 4, and 2 mm. lenses. This suggests that there are spirals of ascending and decending orders of size. This observation is confirmed to some extent by figure 3 which shows spirals of two orders of size.

Spirals of ascending and descending orders of size may be the primordial structural basis of the coordination of functions in living systems.

## INDICATIONS OF SPIRAL STRUCTURE IN POTATO STARCH GRAINS AND CRYSTALS OF CANE SUGAR

ALSBERG (1) apparently quoting MEYER, HOPFF and MARK, states that starch chain molecules are perhaps spirally wound. He also quotes NÄGELI as reporting spirally twisted starch granules.

The writer has examined the starch grains of the potato by bringing a glass slide into contact with a freshly cut potato and allowing the starchy film to dry in air for a few days. In preparations of this kind the material of the starch grains, which were sufficiently transparent, showed series of curves and crescents arranged in such a way as to indicate that they formed parts of closely wound spirals.

Crystals of cane sugar were spread on glass slides, which were slightly moistened with water, and allowed to dry. Some of the crystals which could be examined microscopically showed dark internal patterns indicative of denser areas of spiral formation.

Spiral structure in parts of the cellulose walls of the onion has already been mentioned (see also fig. 2). According to FARR (**4**) cellulose fibrils arranged in cross spirals have become familiar objects in published illustrations of the swollen membranes of cotton fibres.

## SPIRAL SYSTEMS AND INTEGRATION

The widespread occurrence of spiral systems in living protoplasm leads one to inquire if they can be correlated with any functions of the organism. The short spirals such as those in chromosomes and cytoplasm are suggestive of the metallic spirals used in physics as models of short solenoids. When an electric current is passed through these short metallic spirals they exhibit a characteristic magnetic field similar to that of bar magnets. It is possible that in living systems the proteinaceous spirals may possess magnetic fields comparable to those of short solenoids and bar magnets.

The repulsive and attractive movements of chromosomes in mitosis and meiosis are strongly suggestive of magnetic effects. In mitosis the repulsive effects may be produced by electric currents flowing in the same direction in similarly coiled chromatids. In meiosis the attractive effects which produce pairing of homologous chromosomes may be brought about by electric currents flowing in oppositely coiled chromosomes. The work of LUND and his co-workers (**10**), for example, shows that there are electric currents in living organisms. LUND and his associates have also shown that these electric currents are dependent upon cell respiration. According to WARBURG (**15**), cell respiration is a capillary-chemical process which takes place on the surface of the solid cell constituents. As the spirals in protoplasm constitute its principal disperse phase, it is to be expected that the surfaces of the spirals would be the seat of cell respiration, and consequently the loci of electric potentials. Another significant fact is the participation of iron compounds (haemin compounds) in cell respiration. It is generally recognized that the metals themselves are electric conductors of the first class.

In view of the preceding considerations it is suggested that the spirals in protoplasm may be activated by electric currents and possess magnetic fields

comparable to those of bar magnets. If this is so, the positions of the spirals in space and their relationship to each other would be determined by lines of force connected with the spirals themselves. Such an arrangement would represent a dynamic system with considerable possibilities for the fundamental explanation of structures and functions of organisms on an electromagnetic basis.

Integration or the harmonious functioning of the organism as a unit may depend upon protoplasmic spiral systems as the loci of electric potentials and the seat of electromagnetic fields.

## Summary

In extending observations on the spiral structure of protoplasm, photomicrographs were taken of the living protoplasm in the epidermis of the bulb scale of the onion. The photomicrographs show spiral structure in the protoplasm, in the oil bodies suspended in the protoplasm, and in parts of the cellulose walls.

Spiral structure in cellulose walls and oil bodies and indications of spiral structure in potato starch grains and crystals of cane sugar suggest that this type of structure may also be characteristic of protoplasmic products.

In parts of the photomicrographs there are indications of an orderly arrangement of the spirals into systems. The filament composing some of the spirals is shown to be coiled into spirals of two orders of size.

Spirals of ascending and descending orders of size may be the primordial structural basis of the coordination of functions in living systems.

It is suggested that the proteinaceous spirals in protoplasm may be activated by electric currents and possess magnetic fields comparable to those of short solenoids and bar magnets. If this is so, the position of the spirals in space and their relationship to each other would be determined by lines of force connected with the spirals themselves. Such an arrangement would represent a dynamic system with considerable possibilities for the fundamental explanation of structures and functions of organisms on an electromagnetic basis.

Integration or the harmonious functioning of the organism as a unit may depend upon protoplasmic spiral systems as the loci of electric potentials and the seat of electromagnetic fields.

Botanic Gardens
Brisbane, Australia

## LITERATURE CITED

1. Alsberg, C. L. Structure of the starch granule. Plant Physiol. **13**: 295–330. 1938. (See p. 307.)
2. Andrews, F. M. Oil bodies in plants. Abstr. and Titles of Papers, 15th Ann. Meeting, Amer. Society Plant Physiol., p. 12, 1938.

3. CZAPEK, F. Biochemie der Pflanzen, Auf. 3, Bd. 3, p. 190. Fischer, Jena. 1925.
4. FARR, W. K. The microscopic structure of plant cell membranes in relation to the micellar hypothesis. Jour. Phys. Chem. **42**: 1113–1124. 1938.
5. FRANCIS, W. D. The crystalline character of living material. Science n. s. **87**: 16. 1938.
6. ————. The spiral structure of protoplasm. Science n. s. **89**: 127. 1939.
7. KOSHY, T. K. Number and behaviour of chromosomes in *Aloe litoralis*. Ann. Bot. n. s. **1**: 52–58. 1937.
8. KUWADA, Y., and NAKAMURA, T. Behaviour of chromonemata in mitosis. VI. Metaphasic and anaphasic longitudinal split of chromosomes in the homotype division in pollen mother cells in *Tradescantia reflexa*. Cytologia **6**: 314–319. 1935.
9. LEPESCHKIN, W. W. Kolloidchemie des Protoplasmas. p. 146. J. Springer, Berlin. 1924.
10. LUND, E. J., and BUSH, M. Electric correlation potentials in the leaf of *Bryophyllum*. Plant Physiol. **5**: 491–508. 1930.
11. MOLISCH, H. Mikrochemie der Pflanze, 2 Auf. p. 139. Fischer, Jena. 1921.
12. SCARTH, G. W. The structural organization of plant protoplasm in the light of micrurgy. Protoplasma **2**: 189–205. 1927. (See p. 194.)
13. SEIFRIZ, W. Protoplasm. fig. 119. McGraw-Hill, New York. 1936.
14. ————. Recent contributions to the theory of protoplasmic structure. Science n. s. **88**: 21–25. 1938.
15. WARBURG, O. Physikalische Chemie der Zellatmung. Biochem. Zeitschr. **119**: 134–166. 1921.

# RELATION BETWEEN QUANTITY OF CHLOROPHYLL AND CAPACITY FOR PHOTOSYNTHESIS

ROBERT EMERSON, LOWELL GREEN, AND J. LEYDEN WEBB

(WITH ONE FIGURE)

Seeking evidence as to the number of chlorophyll molecules concerned in the reduction of one molecule of carbon dioxide, EMERSON and ARNOLD (3) measured the maximum amount of carbon dioxide which could be reduced by a measured quantity of cells, under conditions designed to make photosynthesis dependent only on the amount of chlorophyll present, and independent of other factors, both internal and external. The limiting effect of chemical processes in the cell under saturating external conditions was avoided by illuminating with extremely short flashes of light (about $10^{-5}$ seconds) and allowing sufficient dark time (about one-tenth of a second) between flashes so that the chemical processes or Blackman reaction could run to completion between light flashes. The intensity of the flashes was so great that higher intensities resulted in no increase in carbon dioxide assimilation. With light flashes as short as $10^{-5}$ sec., it was believed impossible for a significant number of individual chlorophyll molecules to act more than once per flash in the reduction of carbon dioxide. Under these conditions it was anticipated that chlorophyll would be the only factor limiting the amount of carbon dioxide reduced per flash. The number of chlorophyll molecules should limit the energy available for carbon dioxide reduction after each flash. The ratio ($\rho$) of number of chlorophyll molecules present to number of carbon dioxide molecules reduced per flash was found to remain approximately constant, even when the chlorophyll content per unit volume of cells was varied several fold. Unexpectedly high values were obtained for $\rho$, showing that the cells contained two or three thousand times as much chlorophyll as the maximum amount of carbon dioxide which they could reduce per flash of light.

Calculations made by GAFFRON and WOHL (7) show that essentially the same relationship prevails in continuous light. They compared the maximum rate of carbon dioxide reduction with the chlorophyll content for a variety of material, including leaves as well as suspensions of algal cells. Taking the average time required for the completion of the Blackman reaction in Chlorella from the results of EMERSON and ARNOLD, they found that assimilating cells in general contain over two thousand times as much chlorophyll as seems necessary to account for the highest observed rates of photosynthesis.

The measurements of EMERSON and ARNOLD were made only with *Chlorella pyrenoidosa*, but ARNOLD and KOHN (1) later reported measure-

ments with six species, representing four phyla. Their results, and the calculations of GAFFRON and WOHL, have led to the proposal that chlorophyll exists in the cell in some sort of units, each containing some 2000 chlorophyll molecules, such that when a quantum of light is absorbed by any member of a particular unit, the energy may become available for the reduction of a carbon dioxide molecule associated with the unit (*cf.* ARNOLD and KOHN, **1**; GAFFRON and WOHL, **7**; WOHL, **8, 9, 10**). Some of ARNOLD and KOHN's values for $\rho$ run up to four and five thousand, suggesting that $\rho$ may be subject to some variation. Nevertheless, they regard its relative constancy as evidence for the existence of chlorophyll units. Therefore it seemed to the writers important to find within what limits $\rho$ might vary in a single species, *Chlorella pyrenoidosa*. The experiments we report here show a much greater range in the value of $\rho$ than had been indicated previously, so that hypotheses formulated on the basis of the supposed constancy of $\rho$ may not be tenable. The existence and significance of the chlorophyll unit is now the subject of active discussion (*cf.* WOHL, **8, 9, 10**; also FRANCK and HERZFELD, **5**; FRANCK and TELLER, **6**) on which our results appear to have some bearing.

## Experimental

Except for the use of a culture medium saturated with five per cent. carbon dioxide instead of carbonate-bicarbonate mixture as suspending fluid for the cells, our technique was the same as EMERSON and ARNOLD'S. In view of the results reported by EMERSON and GREEN (**4**), who compared the behavior of photosynthesis in carbonate mixtures and in phosphate buffers, we thought it possible that the phenomena in flashing light might depend in some respect on the pH or on the method of carbon dioxide provision. In a culture medium saturated with five per cent. carbon dioxide, photosynthesis is unaffected by considerable variations in both pH and carbon dioxide concentration.

The carbon dioxide assimilation during flashing light is of the same order of magnitude as the respiration, so the correction applied for respiration is of the utmost importance. Respiration measurements were made in darkness, between the exposures to flashing light. Separate experiments on respiration indicated that the small amount of assimilation during flashing light was without measurable influence on the subsequent rate of respiration. As in all measurements of photosynthesis, it remains possible that the respiration is higher during illumination so that the use of a subsequent respiration measurement to compute photosynthesis may lead to a value lower than that actually attained by the organism.

The chlorophyll content was determined by extracting aliquot samples of cells with hot methyl alcohol, and measuring the extinction spectrophotometrically for the Neon line 6598 Å. The same method was used by EMER-

SON and ARNOLD, and by ARNOLD and KOHN. It has been standardized with weighed samples of Chlorella chlorophyll (EMERSON and ARNOLD, **3**) and with chlorophyll prepared from higher plants (ARNOLD and KOHN, **1**) and the values obtained were practically identical.

The accuracy of the method depends on several factors besides the precision of the standardization. Chief among these are the completeness of extraction of the chlorophyll from the cell samples, the freedom of the extract from other substances which absorb appreciably at 6598 Å, and the uniformity of the ratio of the two chlorophyll components. While we cannot state quantitatively how closely these requirements are fulfilled, it seems conservative to say that the error in the analyses is probably less than ten per cent. Since we are concerned here with variations of several fold in the value of $\rho$, such an error in the chlorophyll determinations would not alter our conclusions.

Most of the photosynthesis measurements were made at 25° C., with a frequency of 20 flashes per second, making the dark time between flashes 0.05 sec. According to EMERSON and ARNOLD, at 25° this dark time is sufficient for the completion of the dark processes after each flash. Some of our experiments suggest that cells from young cultures grown over bright light may require a longer dark period. If this proves to be the case, then the

TABLE I

THE RATIO $\rho$ IS SHOWN FOR CULTURES OF VARIOUS AGES, GROWN OVER THREE DIFFERENT SOURCES OF ILLUMINATION. ALL CULTURES WERE GROWN AT 17° C. ± 1°. MEASUREMENTS OF $\rho$ WERE MADE AT 25° C.

| CURVE | LIGHTING CONDITIONS FOR GROWTH OF CULTURES | AGE AT TIME OF HARVESTING | DENSITY AT TIME OF HARVESTING | $\rho$, MOLES OF CHLOROPHYLL / MOLES $CO_2$ REDUCED PER FLASH |
|---|---|---|---|---|
| | | *days* | *mm.³ per ml. of culture* | |
| A | Four forty-watt incandescent lamps 10 cm. from culture flasks, and Corning "Noviol C" filter, transmitting only wave lengths longer than 480 mμ | 2 | 0.1 | 3,750 |
| | | 4 | 0.5 | 6,180 |
| | | 8 | 1.6 | 11,120 |
| B | Four forty-watt incandescent lamps 10 cm. from culture flasks, no filter. | 4 | 0.6 | 3,920 |
| | | 9 | 1.6 | 6,850 |
| | | 17 | 2.2 | 9,720 |
| C | Single forty-watt incandescent lamp, 15 cm. from culture flasks. | 15 | 0.2 | 6,650 |
| | | 21 | 0.5 | 11,000 |
| | | 29 | 0.7 | 14,500 |

values of $\rho$ shown in table I may have to be reduced somewhat for the younger cultures.

The ratio $\rho$ was determined for cells from cultures grown for different periods of time and with different conditions of illumination. In all cases it was found to increase with increasing age of the cultures, but the rate of increase and the value at any given age depended on the illumination used for growing the cultures. Much experimental work has been done on the influence of wave length and intensity of light on the changes in chlorophyll content and photosynthetic capacity with age of culture. The action of these factors is obscured by the increasing selective absorption of light by the cells in a growing culture. This is minimal in a young culture which contains so few cells that light passes through the suspension almost unchanged. But as the culture grows older and increases in density, certain wave lengths are absorbed more strongly than others, and the light which has passed through the peripheral parts of the suspension reaches the cells in the interior greatly diminished in intensity and changed in wave length distribution. The cells are circulated by shaking the cultures at regular intervals, and by the slow stream of five per cent. carbon dioxide in air which bubbles through continuously, so that all are exposed from time to time to the entire range of illumination prevailing within the culture. The changing environment inevitably associated with this simple technique renders it difficult to separate and analyze the influence of the factors in question. While it is clear that the age of the culture, the wave length distribution, and light intensity used for growing the culture all have profound effects on the photosynthetic apparatus, a separation of these effects probably requires more elaborate culture technique. Consequently the present paper deals only with variations in $\rho$, without attempting to trace the influence of culture conditions on the more fundamental internal factors upon which $\rho$ probably depends.

Figure 1 shows three curves for the value of $\rho$ plotted against age of culture for cultures grown under different conditions of illumination. The conditions of illumination for the three curves, as well as the density of each culture at the time of harvest, the age, and the value of $\rho$, are given in table I. The lighting used for the cultures for curves A and B was identical except for the use of a filter[1] for the "A" cultures which transmitted only wave lengths longer than about 480 mμ. The cultures for curve A were therefore deprived of blue and violet light, while the cultures for curve B were exposed to the full spectrum of the incandescent lamps. Comparison of the densities for equal ages of the A and B series in table I shows that the removal of the blue and violet leaves the rate of growth practically unchanged, although for corresponding densities $\rho$ is always higher for the cells grown without blue and violet light.

[1] Corning "Noviol C" filter.

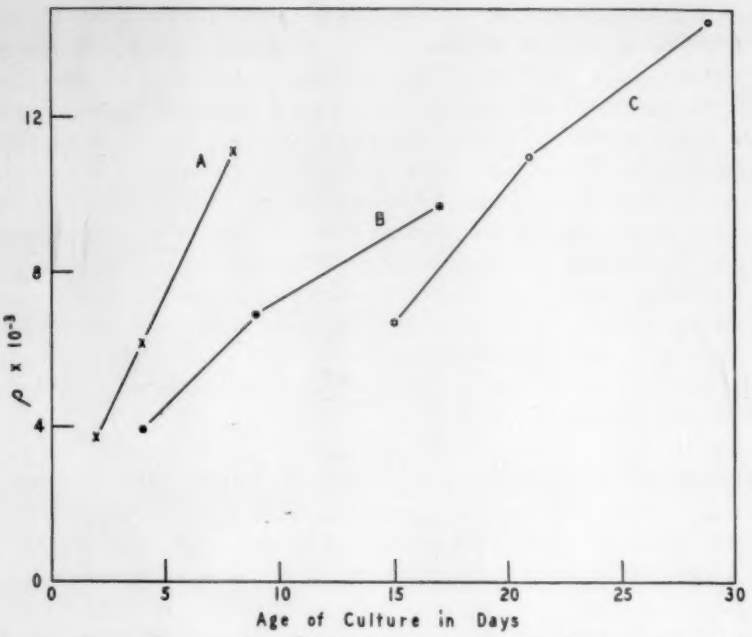



FIG. 1. Three curves showing the influence of age of culture on the value of $\rho$. The illumination for growing the cultures for curve B was from four forty-watt incandescent lamps spaced close together in a square, and 10 cm. distant from the bottoms of the culture flasks. The same lighting arrangement was used for the cultures for curve A, but in this case the light was passed through a glass color filter transmitting only wave lengths longer than about 480 mμ. The cultures for curve C were grown over a single 40-watt incandescent lamp at a distance of 15 cm.

Curve C, made with a series of cultures grown at a very low light intensity, shows higher values of $\rho$ for corresponding culture densities, or lower values for corresponding ages.

## Discussion

Our results indicate that the value of $\rho$ regularly increases with the age of the culture, and is influenced by the lighting conditions used for growth. None of the values reported here are as low as the highest values of EMERSON and ARNOLD (3), but no attempt was made to duplicate their culture conditions. Their primary purpose was to obtain a wide range of chlorophyll concentrations. This they achieved by growing cultures over neon and mercury sign-lighting tubes. The approximate constancy of $\rho$ over a wide range of chlorophyll concentrations seems remarkable, but may be attributable in part to the fact that their cultures were regularly harvested as soon as they had reached a density which provided adequate amounts of material for the measurements, without reference to age.

It might be supposed that the increase in $\rho$ with age of culture indicates an increasing proportion of inactive or moribund cells which still retain their cholorophyll. This would be consistent with the opinion of ARNOLD and KOHN that in all active cells there exists a chlorophyll unit of approximately constant size. There is no reason however, for thinking that our cultures contained increasing quantities of inactive cells. The decline in growth rate with age indicated by the figures for culture density in table I is no more than would be anticipated from the increasing competition for light and nutrients with increasing population density. Differential centrifuging failed to reveal the presence of more and less active fractions among the cell population. We regard it as unlikely that there are appreciable quantities of inactive cells present in cultures during periods of rapid growth, although table I shows that this rapid growth may be accompanied by a sharp increase in the value of $\rho$.

It seems more probable that a chlorophyll unit within the meaning of ARNOLD and KOHN does not exist, and that the large amount of chlorophyll present in comparison with the capacity to reduce carbon dioxide must be explained in other terms. FRANCK and HERZFELD (**5**), and FRANCK and TELLER (**6**) have found it difficult to reconcile the existence of such a unit with modern concepts concerning energy transference between large molecules. EMERSON (**2**) has suggested that the amount of carbon dioxide which can be reduced per flash of light may not depend upon the amount of chlorophyll, but may be a measure of some other substance taking part in photosynthesis. For example, it is possible that assimilating cells contain a limited amount of some substance with which carbon dioxide must combine before it can undergo reduction. Carbon dioxide saturation in flashing light would then be a measure of the full capacity of this substance for combining with carbon dioxide. The fact that $\rho$ is always large would mean that the amount of chlorophyll always greatly exceeds the amount of this proposed substance. Other catalysts or enzymes present in small amount could also account for the observed phenomena, provided they could set a fixed limit to the yield per flash under saturating conditions. The best obtainable yield per flash appears to be always far short of what might be expected from the amount of chlorophyll present.

## Summary

During photosynthesis in flashing light under optimum external conditions, the ratio of moles of chlorophyll present to moles of carbon dioxide reduced per flash is not a constant in *Chlorella pyrenoidosa* cells, but depends on conditions of previous growth, increasing sharply with age of culture and varying with color and intensity of culture illumination. Thus the maximum amount of carbon dioxide reducible per flash is not directly

related to the amount of chlorophyll but depends on some other internal factor.

CALIFORNIA INSTITUTE OF TECHNOLOGY
PASADENA, CALIFORNIA

## LITERATURE CITED

1. ARNOLD, WILLIAM, and KOHN, HENRY I. The Chlorophyll Unit in Photosynthesis. Jour. Gen Physiol. **18**: 109–112. 1934.
2. EMERSON, ROBERT. Photosynthesis. Ann. Rev. Biochem. **6**: 535–556. 1937.
3. ————, and ARNOLD, WILLIAM. The photochemical reaction in photosynthesis. Jour. Gen. Physiol. **16**: 191–205. 1932.
4. ————, and GREEN, LOWELL. Effect of hydrogen-ion concentration on Chlorella photosynthesis. Plant Physiol. **13**: 157–168. 1938.
5. FRANCK, JAMES, and HERZFELD, K. F. An attempted theory of photosynthesis. Jour. Chem. Phys. **5**: 237–251. 1937.
6. ————, and TELLER, E. Migration and photochemical action of excitation energy in crystals. Jour. Chem. Phys. **6**: 861–872. 1938.
7. GAFFRON, HANS, and WOHL, KURT. Zur Theorie der Assimilation. Naturwiss. **24**: 81–107. 1936.
8. WOHL, KURT. Zur Energiebilanz der Kohlensäureassimilation. Zeitschr. physikal. Chem. (B) **31**: 152–156. 1935.
9. ————. Zur Theorie der Assimilation, I. Die Theorie der Assimilationseinheit. Zeitschr. physikal. Chem. (B) **37**: 105–121. 1937.
10. ————. Zur Theorie der Assimilation, IV. Zum Mechanismus der Assimilationseinheit. Zeitschr. physikal. Chem. (B) **37**: 186–208. 1937.

# EXPERIMENTS ON PHOTOPERIOD IN RELATION TO THE VEGETATIVE GROWTH OF PLANTS[1]

JAMES BONNER

## Introduction

It has long been known that relative length of day and night effects the vegetative as well as the reproductive activity of plants. Since the appearance of the paper of GARNER and ALLARD (**7**) which first dealt with this subject, abundant factual material has accumulated relating to the influence of photoperiod on such varied processes as shoot elongation, leaf size, accumulation of dry weight and root growth (**6, 10**). It has been frequently, although not invariably, found that vegetative growth as measured by one or more of these criteria, is greater in long days than in short days irrespective of the reproductive behavior of the species in question. The promotive effect of long daily photoperiods on vegetative growth does not appear to be directly attributable to increased duration of $CO_2$ assimilation (**11**). Plants grown under conditions of long day made up of a short photoperiod of natural light supplemented by light of exceedingly low intensity exhibit the vegetative vigor typical of plants on a long day rather than that of plants on a short day regime (**13**).

It is known that the reproductive response of photoperiod sensitive plants is to be attributed to the production of a specific hormone (or hormones) in the leaves under conditions of suitable day length (**8, 9**). The production of this particular hormone or hormones would appear to depend on the length of day to which the leaves are subjected. It seems logical to inquire in how far the vegetative responses of plants to varying photoperiods are also attributable to the influence of day length on the production of the several hormones which regulate vegetative growth. In the present paper evidence will be presented to show that the production of vitamin $B_1$, a hormone for the growth of roots (**1**), is affected by the length of day to which the plant is subjected.

It is known that length of day exercises a profound effect on the extent of the root system in many species of plants. Thus WEAVER and HIMMEL (**12**) found that the root system is invariably more extensive under conditions of long photoperiod irrespective of whether the species of plant is long day, short day, or indeterminate with regard to flowering behavior. CRIST and STOUT (**5**) have also shown that the root system of plants grown under short photoperiod may be several times smaller than the root systems of similar plants grown under conditions of long photoperiod. These observa-

[1] Report of work done with the cooperation of the Works Progress Administration; Official Project no. 665–07–3–83, Work Project no. 9809.

tions suggest that the roots of plants grown under short photoperiod receive a limited amount of one or more of the substances required for root growth and supplied by the above ground portion of the plant.

## Materials and methods

Plants were grown in the greenhouse in the manner previously described (**2**) in washed quartz sand contained in 2-gallon glazed crocks provided with drainage. They were supplied on alternate days with SHIVES $R_2S_5$ nutrient (including the minor elements Mn, Cu, Zn, B, Mo, and Fe) and the crocks flushed out with tap water on the intervening days. In each experiment one half of the plants were supplied with vitamin $B_1$ at the rate of 0.01 mg. per liter of nutrient solution. This concentration has been found in earlier experiments (**2**) to be non-toxic and promotive to the growth of many species of plants. Conditions of long photoperiod (18 to 20 hours) were maintained by means of supplementary illumination from Mazda lamps, approximately 80 foot-candles intensity at the leaf surface. Conditions of short photoperiod refer to a daily light period of 9 hours (8 A.M. to 5 P.M.).

In each experiment the vitamin $B_1$ content of the leaves (and in some cases of the roots) of the control and experimental plants was determined. This was done by means of the Phycomyces assay whose application to determination of vitamin $B_1$ in plant tissues has been described in detail in an earlier publication (**3**).

## Experimental results

It is shown in table I that of the five species examined in the present experiments, every one contained less vitamin $B_1$ when cultured under a short photoperiod than when cultured under a long photoperiod. Of these spe-

TABLE I

SUMMARY OF DATA RELATIVE TO THE INFLUENCE OF PHOTOPERIOD ON VITAMIN $B_1$ CONTENT OF TOPS OF FIVE SPECIES OF PLANTS, GROWN IN SAND CULTURE AND ON LONG OR SHORT PHOTOPERIOD

| SPECIES | FLOWERING PHOTOPERIODIC BEHAVIOR | VITAMIN $B_1$ CONTENT | | | |
|---|---|---|---|---|---|
| | | SHORT PHOTOPERIOD | | LONG PHOTOPERIOD | |
| | | PER GRAM DRY WEIGHT | PER SHOOT | PER GRAM DRY WEIGHT | PER SHOOT |
| | | γ | γ | γ | γ |
| Xanthium | Short day | 3.4 | 1.72 | 5.2 | 4.20 |
| Cosmos | " " | 4.9 | 0.26 | 5.1 | 0.38 |
| *Brassica alba* | Long day | 4.85 | 0.31 | 6.0 | 0.54 |
| *Brassica nigra* | " " | 2.95 | 0.14 | 3.9 | 0.22 |
| Lycopersicum | Indeterminate | 5.3 | 2.86 | 6.5 | 6.78 |

cies, two were short day with respect to production of flower hormones (that is, were "short day" plants), two were long day with respect to the production of this factor, and one was indeterminate. Nevertheless, all might be termed "long day" with respect to the production of vitamin $B_1$. It appears then justifiable to advance the working hypothesis that the vegetative growth responses of plants to photoperiod may be attributable in part to the decreased production of vitamin $B_1$ under conditions of short day. Support of this hypothesis is supplied by the fact that plants whose vitamin $B_1$ content is low because of maintenance under conditions of short photoperiod, do in certain cases respond more strongly to added vitamin than do parallel plants maintained under conditions of long photoperiod (table II, III).

## TABLE II

EFFECT OF PHOTOPERIOD AND VITAMIN $B_1$ ON THE GROWTH OF XANTHIUM IN SAND CULTURE

| | SHORT PHOTOPERIOD | | | | | LONG PHOTOPERIOD | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | DRY WEIGHT PER PLANT | | VITAMIN $B_1$ CONTENT | | NUMBER OF PLANTS | DRY WEIGHT PER PLANT | | VITAMIN $B_1$ CONTENT | | NUMBER OF PLANTS |
| | TOPS | ROOTS | TOPS | ROOTS | | TOPS | ROOTS | TOPS | ROOTS | |
| | *mg.* | *mg.* | γ | γ | | *mg.* | *mg.* | γ | γ | |
| Control | 510 | 57 | 1.72 | 0.19 | 47 | 810 | 133 | 4.20 | 0.69 | 36 |
| Added vitamin $B_1$ | 855 | 105 | 3.76 | 0.46 | 37 | 1000 | 195 | 7.20 | 1.40 | 41 |

## TABLE III

EFFECT OF PHOTOPERIOD AND VITAMIN $B_1$ ON THE GROWTH OF BRASSICA PLANTS IN SAND CULTURE. PLANTS HARVESTED SIX WEEKS AFTER PLANTING

| SPECIES | SHORT PHOTOPERIOD | | LONG PHOTOPERIOD | |
|---|---|---|---|---|
| | DRY WEIGHT PER PLANT | VITAMIN $B_1$ CONTENT | DRY WEIGHT PER PLANT | VITAMIN $B_1$ CONTENT |
| | *mg.* | γ | *mg.* | γ |
| *B. alba* | | | | |
| Control | 64 | 0.31 | 90 | 0.54 |
| Added vitamin $B_1$ | 290 | 2.25 | 193 | 3.05 |
| *B. nigra* | | | | |
| Control | 48 | 0.14 | 57 | 0.22 |
| Added vitamin $B_1$ | 175 | 1.01 | 136 | 0.87 |

*Xanthium pennsylvanicum* (cocklebur) is a short day plant and flowers if maintained under daily photoperiods shorter than 15.5 hours. In the present experiments, the seedling plants were maintained for 3 weeks under

conditions of long (18-hour) photoperiod. They were then divided into four equal lots and two lots transferred to conditions of a short (9-hour) photoperiod. One lot of those on each photoperiod received nutrient solution with added vitamin $B_1$; the other 2 lots received only nutrient solution. At the end of 4 weeks, when the plants were harvested, those maintained on long photoperiod were strictly vegetative while those maintained on short photoperiod possessed flowers and young fruits. Table II shows that of the control plants, those maintained under short photoperiod formed only 61 per cent. of the dry weight formed by those on long photoperiod, and that the dry weight of roots produced on short photoperiod was less than half that produced on long photoperiod. The plants maintained on short photoperiod and supplied with vitamin $B_1$, however, actually exceeded the long photoperiod control plants in total dry weight and produced 81 per cent. of the dry weight formed by the long photoperiod vitamin $B_1$ plants. The increased response to vitamin $B_1$ under conditions of short photoperiod is particularly striking in the case of the root system. The roots of the vitamin $B_1$ treated plants were almost 100 per cent. heavier than those of the control plants under conditions of short photoperiod and were only 44 per cent. heavier than the control roots under conditions of long photoperiod. Table II shows, then, that Xanthium plants on short photoperiod which contain relatively little vitamin $B_1$, respond more vigorously to addition of vitamin $B_1$ (with increased dry weight deposition) than do the plants on long photoperiod which contain relatively much vitamin $B_1$.

*Brassica alba* and *Brassica nigra* grown from unvernalized seed behave as long day plants. In the present experiments two lots of each species were maintained on long photoperiod and two lots of each species on short photoperiod. At the expiration of 6 weeks when the plants were harvested, those on long photoperiod were in full bloom, while those maintained continuously on short photoperiod were vegetative. Table III shows that with both species, less dry weight was accumulated (in the control series without vitamin $B_1$) under short photoperiod than was accumulated under long photoperiod. In each case also considerably less vitamin $B_1$ was found in the plants under short photoperiod than was found in the plants under long photoperiod. With both species the percentage increase in dry weight under the influence of vitamin $B_1$ is greater under short photoperiod than under long photoperiod.

It has been shown by ČAJLACHJAN and ŽDANOVA (**4**) that more auxin is produced by plants maintained under long photoperiods than by similar plants maintained under short photoperiods. In the present experiments it was found that Xanthium grown under long photoperiod contained considerable amounts of auxin,[2] but when grown under conditions of short

[2] Auxin determinations by Dr. J. VAN OVERBEEK.

photoperiod it contained no appreciable amount of auxin. The production of auxin by the plant appears to be affected by photoperiod just as is the production of vitamin $B_1$. It is to be expected that not only the formation of auxin and vitamin $B_1$ but also the formation of other growth factors may be affected by the daily photoperiod to which the plant is subjected. Under the conditions of the present experiments it would appear that production of vitamin $B_1$ is a particularly limiting factor in the case of Xanthium and of Brassica grown under conditions of short photoperiod. In other cases, other factors may be even more greatly affected than vitamin $B_1$. This may be the case with Cosmos whose response to added vitamin $B_1$ is less under short day conditions than under conditions of long day (table IV) and with tomato which does not respond to added vitamin $B_1$ either

TABLE IV

EFFECT OF PHOTOPERIOD AND VITAMIN $B_1$ ON THE GROWTH OF COSMOS GROWN IN SAND CULTURE. PLANTS HARVESTED FOUR WEEKS AFTER APPEARANCE OF FIRST LEAF

| | SHORT PHOTOPERIOD | | | | LONG PHOTOPERIOD | | | |
|---|---|---|---|---|---|---|---|---|
| | DRY WEIGHT PER PLANT | | NUMBER OF PLANTS | VITAMIN $B_1$ PER SHOOT | DRY WEIGHT PER PLANT | | NUMBER OF PLANTS | VITAMIN $B_1$ PER SHOOT |
| | TOPS | ROOTS | | | TOPS | ROOTS | | |
| | *mg.* | *mg.* | | γ | *mg.* | *mg.* | | γ |
| Control | 53 | 9.2 | 90 | 0.26 | 74.5 | 15.0 | 120 | 0.38 |
| Added vitamin $B_1$ | 67 | 18.0 | 60 | 0.42 | 135.0 | 26.0 | 90 | 0.86 |

under long photoperiod or short photoperiod, although growth is decreased under the latter condition (table V).

TABLE V

EFFECT OF PHOTOPERIOD AND VITAMIN $B_1$ ON THE GROWTH OF TOMATO PLANTS IN SAND CULTURE. PLANTS HARVESTED THREE WEEKS AFTER APPEARANCE OF SECOND LEAF

| | SHORT PHOTOPERIOD | | | | LONG PHOTOPERIOD | | | |
|---|---|---|---|---|---|---|---|---|
| | DRY WEIGHT PER PLANT | | NUMBER OF PLANTS | VITAMIN $B_1$ PER SHOOT | DRY WEIGHT PER PLANT | | NUMBER OF PLANTS | VITAMIN $B_1$ PER SHOOT |
| | TOPS | ROOTS | | | TOPS | ROOTS | | |
| | *mg.* | *mg.* | | γ | *mg.* | *mg.* | | γ |
| Control | 540 | 70 | 10 | 2.86 | 1050 | 184 | 10 | 6.78 |
| Added vitamin $B_1$ | 540 | 70 | 10 | 3.18 | 1260 | 248 | 10 | 5.67 |

## Discussion

In an earlier paper (3) it has been shown that the leaves of the different species of plants vary greatly in their content of vitamin $B_1$. Different

species presumably vary then in the amount of vitamin $B_1$ which they are able to synthesize. It has also been shown that of these various species, those which synthesize relatively large amounts of vitamin $B_1$ do not respond to the addition of this substance with increased growth, while those species which produce relatively little vitamin $B_1$ do respond to its addition with increased growth. The foregoing data show that with both Xanthium and Brassica, the amount of vitamin $B_1$ present in the plant is influenced by the photoperiod to which the plant is subjected. This is not unexpected in view of the fact that vitamin $B_1$ is formed by higher plants in the presence of light (**2**). The data also indicate that with Xanthium and Brassica the growth response to added vitamin is greater under conditions of short photoperiod (low vitamin $B_1$ synthesis) than it is under conditions of long photoperiod (higher vitamin $B_1$ synthesis). In the present experiments the vitamin $B_1$ content of the plant varies with the photoperiod; in the earlier experiments the vitamin $B_1$ content of the plant varied with the species but in both cases, relatively low vitamin $B_1$ content may be correlated with relatively large growth response to added vitamin $B_1$.

## Summary

1. Plants of Xanthium, *Brassica alba*, *Brassica nigra*, Cosmos, and Lycopersicum all contain more vitamin $B_1$ when grown under conditions of long photoperiod (18 hours) than when grown under conditions of short photoperiod (9 hours).

2. Xanthium and Brassica respond to additions of vitamin $B_1$ more strikingly under the conditions of short photoperiod than under those of long photoperiod.

3. It is suggested that the effect of photoperiod on the vegetative growth of plants may be mediated by the effect of photoperiod upon the production, not only of vitamin $B_1$, but also on the production of other growth factors.

William G. Kerckhoff Laboratories of the Biological Sciences
California Institute of Technology
Pasadena, California

## LITERATURE CITED

1. Bonner, J. Vitamin $B_1$ a growth factor for higher plants. Science n.s. **85**: 183–184. 1937.
2. ————, and Greene, J. Vitamin $B_1$ and the growth of green plants. Bot. Gaz. **100**: 226–237. 1938.
3. ————, ————. Further experiments on the relation of vitamin $B_1$ to the growth of green plants. Bot. Gaz. **101**: 491–500. 1939.
4. Čajlachjan, M. Ch., and Ždanova, L. P. Hormones of growth in for-

mation processes. I. Photoperiodism and creation of growth hormones. Compt. Rend. Acad. Sci. U.S.S.R. **19**: 107–111. 1938.

5. CRIST, J., and STOUT, J. Relation between top and root size in herbaceous plants. Plant Physiol. **4**: 63–85. 1929.
6. GARNER, W. "Photoperiodism," in Biological Effects of Radiation, vol. II. 677–713, New York, 1936.
7. ————————, and ALLARD, H. Effect of the relative length of day and night and other factors of the environment on growth and reproduction in plants. Jour. Agr. Res. **18**: 553–606. 1920.
8. HAMNER, K. C., and BONNER, J. Photoperiodism in relation to hormones as factors in floral initiation and development. Bot. Gaz. **100**: 388–431. 1938.
9. MOSKOV, B. S. Photoperiodism and a hypothesis as to hormones of flowering. Compt. Rend. Acad. Sci. U.S.S.R. **15**: 211–214. 1937.
10. MURNEEK, A. E. Biochemical studies of photoperiodism in plants. Univ. Missouri Agr. Exp. Sta. Res. Bull. 268. 1937.
11. TAGEEVA, SOPHIE. Zur Frage des Zusammenhangs zwischen Assimilation und Ertragsfähigkeit. Planta **17**: 758–793. 1932.
12. WEAVER, J., and HIMMEL, W. Relation between development of root system and shoot under long and short day illumination. Plant Physiol. **4**: 435–457. 1929.
13. WITHROW, R. B., and BENEDICT, H. M. Photoperiodic responses of certain greenhouse annuals as influenced by intensity and wave length of artificial light used to lengthen the daylight period. Plant Physiol. **11**: 225–249. 1936.

# OBSERVATIONS ON THE DEVELOPMENT OF CERTAIN CELL-WALL CONSTITUENTS OF FORAGE PLANTS[1]

EMMETT BENNETT

(WITH TWO FIGURES)

## Introduction

In the conventional methods of "proximate analysis," pectic substances, hemicelluloses, and lignin, for the most part, appear in the "nitrogen-free extract" fraction. It is now generally recognized that this method of differentiation gives very little information as to the nature of the substances composing these fractions; hence attempts are being made to ascertain their chemical constitution, nutritional value, precursors, and relationships in the plant. In this way a better understanding of the metabolism and economic value of plants will be obtained.

The changes which the cell-wall constituents undergo during the development of the plant are not definitely known. True chemical relationships cannot be established as yet because the chemical constitution of lignin is not known. Transformation studies have been made, however, and a number of theories have been evolved. The principal theories as summarized by NORMAN (**14**) are:

1. That pectin undergoes transformation to lignin.
2. That the hemicelluloses or certain groups thereof may be converted to lignin.
3. That the polyuronide hemicelluloses are formed from pectin.
4. That all three substances are connected, lignin being formed from pectin through the intermediate stage of polyuronide hemicelluloses.

Most investigations of this kind have dealt with woody tissues, while a few have dealt with grasses, especially cereal grasses. Instances are very few, however, in which transformation studies of these compounds have been made during the growing period of any one species.

This paper presents results of a 2-year study of the content of pectin, hemicelluloses, and lignin in Kentucky blue grass (*Poa pratensis*) and red clover (*Trifolium pratense*) taken at successive stages of growth, with special reference to the formation of lignin.

## Review of literature

The literature is both obscure and contradictory. No attempt is made to give a complete review; instead, the reader is directed to the relatively recent publications by PHILLIPS (**16**) and NORMAN (**14**).

[1] Published as Contribution no. 343 of the Massachusetts Agricultural Experiment Station.

In general, transformation theories assume that pectic substances are eventually replaced by lignin. The intermediate steps, however, are problematical. FUCHS (**10**) assumes that the primary reduction products of the cell-wall constituents, especially pectin, are transformed into phenols, which by further changes, especially condensations, may result in the formation of lignin. EHRLICH (**9**) also considers pectin to be an intermediate product in this synthesis. He assumes, however, that the transformation comes about through enzymatic action involving decarboxylations, dehydrations, and reductions. Other investigators obtained analytical data which indicated that some such change occurs. CANDLIN and SCHRYVER (**7**) note that lignified tissues contain relatively large amounts of lignin and hemicelluloses, with only traces of pectin; non-lignified tissues, on the other hand, contain relatively large amounts of pectins, small amounts of hemicelluloses, and no lignin. MEHTA (**11**) and RITTER (**19**), using pectic solvents, fail to find any pectin in the middle lamella of mature woody tissue. O'DWYER (**15**) suggests that the transformation takes place through the hemicelluloses. This suggestion is supported by BAILEY'S (**3**) work on the middle lamella of Douglas fir in which he found lignin associated with pentosans.

Other investigations have disclosed no definite support for the above transformation theories. ANDERSON (**1**) concludes from his work on black locust wood that pectic substances are laid down early in growth and remain unchanged as the wood ages. A study of the pectic substances in the developing pods of beans and sweet peas by BUSTON (**6**) reveals no direct evidence for the transformation of pectin to lignin. A new and different way of explaining the whole problem is offered by BAILEY (**4**) in which he states that because of the difference in cell structure it is not essential to account for the various ratios of these substances in different plants on the basis of transformation.

## Materials and methods of analysis

Samples of Kentucky blue grass were obtained from the Massachusetts State College Farm at Amherst, Massachusetts, for the years 1935, 1936, and 1937, and of red clover for the years 1936, and 1938. The first clippings of the grass were taken when about three inches tall; subsequent clippings were made every 5 to 8 days through the blooming stage. Clover clippings were not obtained as early in the season but were made in the same way as the grass thereafter. All samples were dried in a forced draft oven at 60–65° C.

The following methods of analysis were used on finely ground samples.

*Ash, nitrogen,* and *total furfural* were determined by the A.O.A.C. (**2**) methods.

*Total pectin.*—All samples were extracted with alcohol prior to an exhaustive extraction with 0.6 per cent. ammonium citrate (**8**).

*Total hemicelluloses.*—The procedure outlined by BUSTON (5) was adopted.

*Lignin.*—A modified SCHWALBE (20) method was used on material which had first been extracted with an alcohol-benzene solution.

## Experimentation

All results obtained are presented in tabular form; those for pectin, hemicelluloses, and lignin are also shown graphically.

TABLE I

PARTIAL PERCENTAGE COMPOSITION OF KENTUCKY BLUE GRASS AT DIFFERENT STAGES OF GROWTH FOR THE YEARS 1935, 1936, AND 1937 ON A DRY MATTER BASIS

| DATE OF SAMPLING | ASH | NITROGEN | TOTAL PECTIN | TOTAL HEMICELLULOSES | LIGNIN | TOTAL FURFURAL |
|---|---|---|---|---|---|---|
| | % | % | % | % | % | % |
| 4/26/35 | 7.05 | 4.06 | 0.67 | 14.24 | 2.86 | 6.25 |
| 5/ 1/35 | 5.93 | 3.35 | 0.78 | 19.12 | 3.53 | 7.56 |
| 5/ 9/35 | 5.91 | 2.70 | 0.86 | 22.58 | 5.06 | 8.86 |
| 5/16/35 | 4.82 | 2.03 | 0.74 | 26.44 | 6.33 | 10.48 |
| 5/21/35 | 5.10 | 1.87 | 0.90 | 26.00 | 6.88 | 10.20 |
| 5/27/35 | 4.25 | 1.50 | 0.96 | 27.76 | 7.75 | 11.97 |
| 5/ 8/36 | 7.30 | 3.65 | 0.90 | 18.07 | 4.50 | 8.72 |
| 5/13/36 | 7.00 | 3.39 | 0.93 | 19.51 | 5.86 | 8.48 |
| 5/20/36 | 6.58 | 2.66 | 0.95 | 20.22 | 6.83 | 9.78 |
| 5/25/36 | 5.80 | 2.20 | 0.59 | 20.47 | 7.62 | 11.16 |
| 6/ 1/36 | 4.96 | 1.66 | 0.62 | 21.55 | 8.50 | 11.20 |
| 6/ 5/36 | 4.85 | 1.57 | 0.52 | 21.01 | 8.45 | 11.77 |
| 6/10/36 | 4.46 | 1.21 | 1.11 | 25.08 | 10.65 | 12.56 |
| 5/16/37 | 7.31 | 3.34 | 0.87 | 20.41 | 3.17 | 9.59 |
| 5/21/37 | 7.47 | 2.92 | 0.85 | 21.36 | 3.79 | 9.79 |
| 5/26/37 | 6.96 | 2.54 | 0.74 | 22.15 | 4.78 | 10.78 |
| 6/ 2/37 | 6.56 | 2.21 | 0.78 | 24.73 | 6.40 | 11.70 |
| 6/ 9/37 | 6.17 | 1.78 | 0.83 | 25.23 | 6.83 | 11.72 |

### TOTAL ASH

In general, the percentage content of ash in both plants decreased as the season progressed. The percentage of ash in the early part of the season was consistently greater in red clover. Both these observations are in agreement with similar work of others.

### TOTAL NITROGEN

The percentage of total nitrogen in both plants followed practically the same seasonal trend as that of the ash. Red clover contained a slightly greater percentage of nitrogen than did Kentucky blue grass.

## TABLE II

PARTIAL PERCENTAGE COMPOSITION OF RED CLOVER AT DIFFERENT STAGES OF GROWTH FOR THE YEARS 1936 AND 1938 ON A DRY MATTER BASIS

| DATE OF SAMPLING | TOTAL ASH | TOTAL NITROGEN | TOTAL PECTIN | TOTAL HEMICELLULOSES | LIGNIN | TOTAL FURFURAL |
|---|---|---|---|---|---|---|
| | % | % | % | % | % | % |
| 5/ 8/36 | 10.22 | 4.80 | 5.61 | 6.93 | 4.82 | 4.85 |
| 5/13/36 | 9.23 | 4.11 | 5.62 | 8.02 | 5.11 | 5.12 |
| 5/20/36 | 9.09 | 3.71 | 5.02 | 8.45 | 5.40 | 5.58 |
| 5/25/36 | 5.77 | 3.01 | 5.22 | 9.00 | 6.18 | 6.12 |
| 6/ 1/36 | 4.94 | 2.97 | 4.93 | 9.86 | 6.83 | 5.50 |
| 6/ 5/36 | 4.62 | 2.63 | 5.13 | 11.19 | 9.20 | 6.51 |
| 6/10/36 | 4.31 | 2.69 | 5.53 | 11.38 | 9.48 | 6.58 |
| 5/14/38 | 10.85 | 3.63 | 7.19 | 4.30 | ..... | 5.20 |
| 5/19/38 | 10.37 | 3.53 | 8.39 | 5.69 | 3.71 | 5.58 |
| 5/25/38 | 9.93 | 3.07 | 8.15 | 5.68 | 3.87 | 6.25 |
| 6/ 1/38 | 9.92 | 2.84 | 8.07 | 6.98 | 4.80 | 6.35 |
| 6/ 9/38 | 8.92 | 2.75 | 7.89 | 7.00 | 5.82 | 6.61 |
| 6/20/38 | 7.72 | 2.43 | 6.99 | 8.48 | 8.32 | 6.81 |

### TOTAL PECTIN

Pectic substances in Kentucky blue grass were present in small and relatively constant amounts. BUSTON (5) working with forage grasses found that they are definitely low in pectin and that the relative amounts of hemicelluloses and pectin are similar to those in wood and straw.

Red clover on the other hand contained relatively large amounts, which showed no definite seasonal trend. The results of the last year, however, showed a slight but insignificant decrease as the plant grew older. A comparison of the pectic content of this plant with that of certain structures

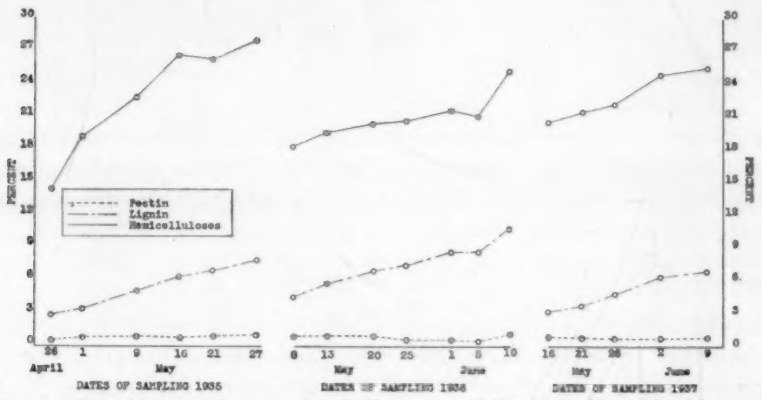



FIG. 1. Percentages of pectin, hemicelluloses, and lignin in Kentucky blue grass at successive stages of growth.

from other plants (**6, 12**) revealed that the amounts present, in 1938 especially, were similar to leaves.

An inspection of figures 1 and 2 will disclose the relative differences in

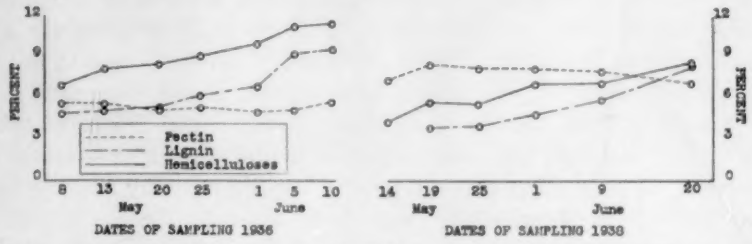



FIG. 2. Percentages of pectin, hemicelluloses, and lignin in red clover at successive stages of growth.

content of pectin of the two plants, and the fact that the percentage content of the last clipping for either plant in every case was practically equal to or greater than that in the first stage of growth. These results do not suggest that a transformation of pectin has taken place. BUSTON (**6**) working with developing pods of beans and sweet peas, obtained results of a similar nature.

### HEMICELLULOSES

With a few minor exceptions the percentage of hemicelluloses in both plants increased as the season advanced. Relatively high percentages of this group were found in both species at a very early stage. The percentage in red clover was somewhat less than half that in Kentucky blue grass and was, in some cases, associated with a similar percentage of pectin. In mature Kentucky blue grass the ratio of hemicelluloses to pectin was about twenty-five to one. These two examples clearly illustrate the ratio of hemicelluloses to pectin that is generally expected in non-lignified and lignified tissue respectively.

### TOTAL FURFURAL

No attempt was made to account for the furfural on the basis of source material. The percentage found, therefore, represents furfural contributed by pentosans, pentoses, and uronic acids, which for the most part came from the hemicelluloses and pectins. In general the percentage of total furfural increased in both species as the season advanced.

### LIGNIN

Lignin, like the hemicelluloses, was present in both species in the earliest stage of growth and increased in content as the season progressed. The maximum values differed but very little between the species. This fact is unusual in view of the large species differences in the percentages of pectin,

and hemicelluloses. Seasonal trends are in general agreement with the reports of others (**13, 17, 18**).

## Discussion

The problem of expressing data obtained from a series of samples representing different ages and sizes, with the hope of illustrating the actual metabolic picture, is difficult. The successful expression of data of this kind on a percentage basis may be masked because other constituents vary in percentage content from time to time; the expression then is one of relative concentration and does not account for the actual quantities involved during growth. A decreasing percentage of a constituent may actually represent an increase in the absolute quantity elaborated. On the other hand, for constituents present in small percentages which remain constant or increase, there can be but little doubt that the quantities increase during the growing process.

In general, the theories involving the formation of lignin are based upon the assumption that hemicelluloses and pectins are labile; hence it is assumed that pectin will eventually disappear. The figures obtained in this investigation show that the pectin content remains relatively constant while other constituents are on the increase. This indicates that pectin is at least formed faster than it is transformed. This indication, together with the fact that red clover contains an amount of pectin in some cases equal to the content of lignin, which in turn is similar in amount to that found in plants having only traces of pectin, is contrary to the above theory.

If hemicelluloses are to be considered precursors of lignin it is quite evident that the percentage of lignin in Kentucky blue grass should have been much higher, or that of red clover much lower. In considering the hemicelluloses as intermediate compounds between pectin and lignin, it will be noticed that the amounts of these substances bear the opposite rather than the expected quantitative relationship to each other.

It is interesting to note that hemicelluloses and lignin were found in definite amounts in the very earliest samples obtained. These observations suggest that the compounds are likely to be active metabolic products rather than products characteristic of maturity in plants.

From the chemical data, no direct relationship between the three substances is apparent. In the absence of cytological data it would seem that the evidence favors BAILEY'S (**4**) explanation; namely, that the various ratios of these substances may be accounted for on the basis of cell structure.

## Summary

A study was made of the ash, nitrogen, pectin, hemicelluloses, and lignin in Kentucky blue grass and red clover at successive stages of growth, with special reference to the formation of lignin.

In general the percentages of ash and total nitrogen decreased throughout the growing season.

The percentages of pectin in both species remained relatively constant during the growth periods. Kentucky blue grass was similar to wood or straw in percentage of pectin, while red clover was comparable to leaves.

The percentage of hemicelluloses increased in both species as the season progressed. The percentage of hemicelluloses in mature Kentucky blue grass was about twenty-five times greater than that of pectin, while in red clover the content of hemicelluloses was sometimes less than that of pectin and never more than about twice as large.

The percentage of lignin in both species increased as the plants grew older. The maximum percentage was about the same for both species.

Approximately the same percentage of lignin was associated with different proportions of pectin and hemicelluloses in the two species.

Lignin and hemicelluloses were found at a very early age.

No direct evidence was obtained for a relationship between pectin, hemicelluloses, and lignin. The results suggest rather that the different proportions of these substances may be attributed to a difference in cell structure.

MASSACHUSETTS AGRICULTURAL EXPERIMENT STATION,
AMHERST, MASSACHUSETTS

## LITERATURE CITED

1. ANDERSON, E., SEIGLE, L. W., KRZNARICH, P. W., RICHARDS, L., and MARTENY, W. W. The isolation of pectic substances from wood. II. Jour. Biol. Chem. **121**: 165–174. 1937.
2. Assoc. Offic. Agr. Chem. Official and tentative methods of analysis. Compiled by the Committee on editing methods of analysis. Third edition. Washington, D. C. 1930.
3. BAILEY, A. J. Lignin in Douglas fir. Ind. & Eng. Chem. Anal. Ed. **8**: 389–391. 1936.
4. BAILEY, I. W. Cell wall structure of higher plants. Ind. & Eng. Chem. **30**: 40–47. 1938.
5. BUSTON, H. W. The polyuronide constituents of forage grasses. Biochem. Jour. **28**: 1028–1037. 1934.
6. ————————. Observations on the nature, distribution and development of certain cell-wall constituents of plants. Biochem. Jour. **29**: 196–218. 1935.
7. CANDLIN, E. J., and SCHRYVER, S. B. Investigations of the cell-wall substances of plants, with special reference to the chemical changes taking place during lignification. Proc. Roy. Soc. (London) B **103**: 365–376. 1928.

8. Conrad, C. M. A biochemical study of the insoluble pectic substances in vegetables. Amer. Jour. Bot. **13**: 531–547. 1926.
9. Ehrlich, F. Über die Chemie des Pektins und seine Beziehungen zur Bildung der Inkrusten der Cellulose. Cellulosechemie **11**: 161–170. 1930. Cited by (**18**).
10. Fuchs, W. M. The chemical nature of lignin. Paper Trade Jour. **102**: 33–36. March 26, 1936.
11. Mehta, M. M. Biochemical and histological studies on lignification. Part II. Histological studies on the polysaccharides and aromatic constituents of the cell wall. Biochem. Jour. **19**: 979–997. 1925.
12. Nanji, D. R., and Norman, A. G. The estimation of the individual pectic substances in nature. Biochem. Jour. **22**: 596–604. 1928.
13. Norman, A. G. A preliminary investigation of the development of structural constituents in the barley plant. Jour. Agr. Sci. **23**: 216–227. 1933.
14. ————. The biochemistry of cellulose, the polyuronides, lignin, etc. Oxford U. Press, London, 1937.
15. O'Dwyer, M. H. Preliminary investigations on the constitution of the hemicelluloses of timber. Biochem. Jour. **22**: 381–390. 1928.
16. Phillips, M. The chemistry of lignin. Chem. Rev. **14**: 103–170. 1934.
17. ————, Davidson, J., and Weihe, H. D. Studies of lignin in wheat straw with reference to lodging. Jour. Agr. Res. **43**: 619–626. 1931.
18. ————, and Goss, M. J. Composition of the leaves and stalks of barley at successive stages of growth, with special reference to the formation of lignin. Jour. Agr. Res. **51**: 301–319. 1935.
19. Ritter, G. L. Distribution of lignin in wood. Ind. & Eng. Chem. **17**: 1194–1197. 1925.
20. Schwalbe, H. Über neue Methode zur Bestimmung des Lignins. Papierfabr. **23**: 174–177. 1925.

# SOIL TEMPERATURE AND GROWTH OF MARQUIS WHEAT

D. J. Wort

(WITH THREE FIGURES)

## Introduction

The development of plants as influenced by soil temperature, as distinct from air temperature, has not been investigated as fully as have growth responses to other environmental factors.

Broadly speaking, roots grow best at lower temperatures than the shoots and they suffer considerably at higher temperatures. Brenchley (**2**) records injury and death of plants from high root temperatures in water cultures. Bushnell (**3**) grew potatoes at different soil temperatures and obtained the highest yield of tubers at 18° C., but maximum top growth occurred at 21° C.

From the time of Sachs's classical experiment in 1860 it has been known that decreased soil temperature results in decreased absorption of water by plants. Sachs (**8**) placed a well-watered tobacco plant in a warm room and surrounded the pot with ice. After a brief period the plant began to wilt, but when the ice was removed and the soil heated it recovered without the addition of water. Kramer (**7**), using a porcelain absorbing surface, determined that increasing soil temperatures (0° C. to 43° C.) materially increased the movement of water from the soil, or in other words, increased the water supplying capacity of the soil.

Transpiration of *Helianthus annuus* varies very little with soil temperatures between 55° F. and 100° F., drops rapidly below 55° F., is reduced to half at 38° F., and approaches zero at 32° F., according to the findings of Clements and Martin (**4**).

Jones (**5**) reports that low soil temperatures are responsible for chlorosis of gardenias. His four conclusions are: 1. color of leaf is affected by soil temperature; 2. size of leaf and rate of growth are related to soil temperature and not to air temperature, leaf size increases with increasing temperatures up to 32° C.; 3. bud set is best obtained by growing plants at high soil temperature and then dropping the temperature to a point where vegetative development is stopped; 4, air temperature is of little importance, and that only in as much as it affects soil temperature.

Soil temperature effects on blooming were investigated by Allen (**1**). He found that the flowering of some varieties of stocks was affected but little by soil temperature; higher temperatures increased the length of time to bloom and the number of flowers per plant of snapdragons. The morphology of wheat seedlings as related to soil temperature was studied by Taylor and McCall (**9**).

JONES *et al.* (6) state that the wheat plant functions best in all stages of its development at relatively low soil temperatures. The favoring influence of low temperature is more marked during the seedling stage. While the wheat (Marquis and Turkey) started germination most promptly at 24° to 28° C., germination was more uniform and stronger plants resulted at low soil temperatures, of about 8° to 10° C. Although the higher soil temperatures stimulated the early development of tops, these too were ultimately stronger at the lower temperatures. The best plant, as measured by stockiness and early maturity, developed at a soil temperature of about 16° C.

## Experimentation

An experiment to determine the response of Marquis spring wheat to various soil temperatures was conducted during the summer of 1937 and repeated with slight modifications and more numerous temperatures during the summers of 1938 and 1939.

### METHOD

Marquis wheat was planted one inch deep in good loam soil in two-gallon porcelain containers. The containers were submerged in water in covered tanks to within 2 inches of their tops. The temperature of the water was thermostatically controlled with the exception of the 22° C. tank. Twenty-two degrees was the lowest temperature that could be obtained by using continuously running water from the water mains and the temperature of the water (hence of the soil in the container) in this tank rose at times to 24° C. and fell to 21° C. Fluctuations of 1° C. in the other tanks were seldom. The dates of planting, harvest, durations of the experiments, number of replicate containers at each soil temperature, number of plants and the soil temperatures used are given in table I.

TABLE I

DATES OF PLANTING, HARVEST, DURATIONS OF EXPERIMENTS, AND SOIL TEMPERATURES USED

| YEAR | DATE OF PLANTING | DATE OF HARVEST | POTS AT EACH TEMPERATURE | TOTAL PLANTS AT EACH TEMPERATURE | DURATION OF EXPERIMENT | SOIL TEMPERATURES °C. |
|---|---|---|---|---|---|---|
| 1937 | July 1 | Aug. 27 | 2 | 10 | 57 days | 22°, 33°, 44° |
| 1938 | July 9 | Aug. 26 | 2 | 10 | 48 " | 22° to 44° in intervals of 2 degrees |
| 1939 | July 3 | Sept. 11 | 5 | 15 | 70 " | 22° to 42° in intervals of 4 degrees |

In 1937 and 1939 the wheat was allowed to grow at a soil temperature of 26° C. for 5 days before the final soil temperatures, given in table I, were established and maintained. In this way the plants later grown at the various soil temperatures were well established. The 1938 temperatures were maintained from the beginning, the grain being sown in soils at these temperatures. The grain failed to germinate at 42° and 44° C., and although resown, the wheat failed to grow. In the 1937 experiment all plants grown at 44° C. died within 35 days.

The temperature in the greenhouse was uncontrolled and rose nearly every day to at least 100° F., falling at night to approximately 70° F.

The pots were given sufficient water once a day to keep the soil at all temperatures moistened to approximately the same degree. More water had to be added to the higher temperature pots than to the lower. Drains from the containers provided constant drainage of excess water.

When the plants were harvested the soil was washed from the roots and the roots then rinsed several times in tap water which removed practically all adhering soil particles. The roots were then rinsed several times in a 5 per cent. sodium chloride solution and finally in distilled water.

## Results and discussion

As mentioned above the wheat failed to grow at soil temperatures of 42° and 44° C. in the 1938 experiment. These temperatures were maintained from the time of sowing. In the 1937 experiment all plants grown at 44° C. died within 35 days.

The data for the three experiments are very similar and those for 1939, being representative, are the only ones tabulated. Significant differences are mentioned in the discussion.

TABLE II

GROWTH AND HARVEST DATA, 1939

| Soil temperature | Average height at 14 days | Average height at 70 days | Average greatest root length at 70 days | Average tillers per plant at 70 days | Average days to head | Average dry weight per plant 70 days | | | Top/Root ratio |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Tops | Roots | Total | |
| °C. | in. | in. | in. | | days | gm. | gm. | gm. | |
| 22 | 10.1 | 25.0 | 12.2 | 4.3 | 65 | 1.61 | 0.40 | 2.01 | 4.0 |
| 26 | 10.2 | 22.5 | 12.1 | 3.5 | 63 | 0.96 | 0.23 | 1.19 | 4.2 |
| 30 | 9.3 | 20.1 | 9.9 | 2.3 | 59 | 0.62 | 0.11 | 0.73 | 5.6 |
| 34 | 8.4 | 16.4 | 4.4 | 1.6 | 54 | 0.21 | 0.03 | 0.24 | 7.0 |
| 38 | 7.0 | 11.5 | 3.3 | 1.3 | 61 | 0.14 | 0.03 | 0.17 | 4.7 |
| 42 | 6.0 | 7.2* | 2.3* | 1.4* | ..... | 0.06* | 0.013 | 0.073 | 4.6 |

* Only 8 of the original 15 plants survived at 70 days.

### Height of plants, root length, and tiller number

The heights of the plants, measured from the soil level to the tip of the longest leaf of the leading tiller, were taken throughout the growing period at weekly intervals.

The curves of the average growth of the plants at each soil temperature are shown in figure 1. The comparative heights of the plants at 14, 21, 28,

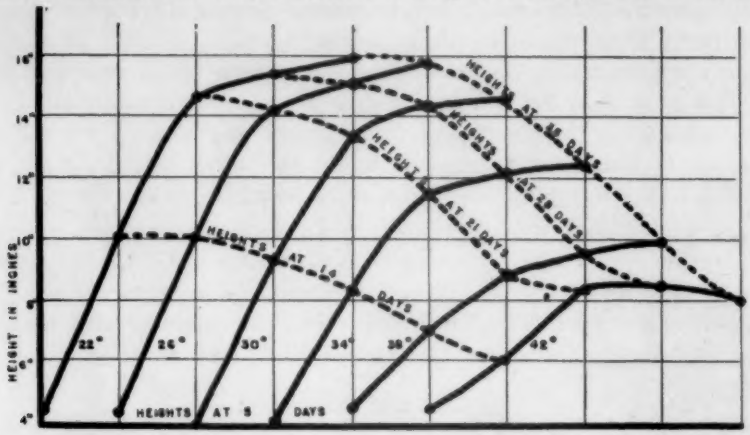



Fig. 1. Curves of the average growth of Marquis wheat from 5 days to 35 days. The solid graphs represent the average growth of plants at the soil temperatures indicated. The broken lines are the height curves at the ages stated.

and 35 days are given by the broken line. The decrease in height of the plants grown at a soil temperature of 42° C. between the age of 28 and 35 days is attributed to considerable death of leaves in this interval. The height of these plants at 70 days was 7.2 inches, whereas the average height at the age of 28 days was 7.47 inches.

Growth of plants at soil temperature of 26° C. for the first 5 days, and then at various soil temperatures (as in 1937 and 1939), produced greater height at the higher temperatures and lesser height at lower temperatures, measured 14 days after planting, compared with plants grown at the various soil temperatures from the start (as in 1938). This treatment also enabled the plants to withstand high soil temperatures for an additional 65 days. The heights at the same temperatures 42 days after planting were very similar in the three experiments, differing by less than an inch in most cases. The differences caused by the initial treatment had been overcome.

The number of tillers decreased from 4.3 at 22° C. to 1.4 at a soil temperature of 42° C., as shown in table II. A corresponding decrease in yield of grain would be expected to follow owing to the reduction in the number of heads.

The length of roots decreased with increasing temperatures, the greatest

decrease occurring between 30° and 34° C. The greatest decrease in stem height occurred between 34° and 38° C. The greater decrease in roots than in stems, relatively, between 30° and 34° C. results in an increased top root ratio at these temperatures, but the later decrease in tops restores the ratio to approximately the same value as at the lower soil temperatures used.

### Weights of tops and roots and top root ratio

Dry weights of tops and roots and total dry weight decreased with increasing temperatures as shown in table II. These values are expressed graphically in figure 2.

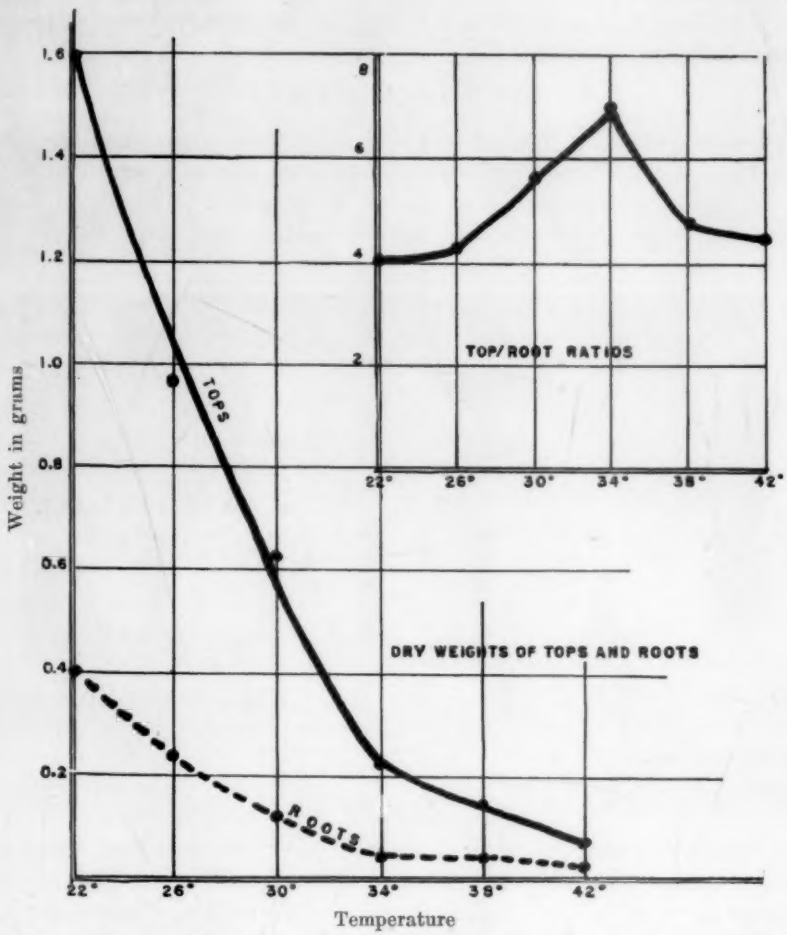



Fig. 2. Dry weights of tops and roots and the top root ratios. The weights are the average per plant, expressed in grams, for plants grown at the soil temperatures indicated.

In the 1939 experiment the top root ratio rose from 4.0 at 22° to 7.0 at 34° C. and fell thereafter to 4.6 at 42° C. This is indicative of a greater comparative reduction in roots than in tops as the soil temperature rises to 34° C. Above this temperature the falling ratio indicates a greater reduction in tops in comparison with roots. The results of the former experiments indicate the same ratio behavior. In 1938 the ratio rose from 4.6 at 22° to 7.1 at 30° C., falling once more to 3.0 at 40° C. It is apparent, therefore, that with increasing soil temperatures the top root ratio increases reaching a maximum at 30°–34° C., and falls again at higher soil temperatures. The findings of these experiments indicate that the general statement found in the literature that roots suffer more than shoots at higher temperatures is true for Marquis wheat only to 30°–34° C. Above these temperatures the reverse holds true.

### Size and color of leaves

Observations as to the leaf size and color were made when the plants were 42 days old. Leaf length, width, and area were at a maximum at 22° C., the lowest temperature used in the experiments. The leaves became visibly lighter in color as the soil temperature increased above 32° C.

Fig. 3. Plants of the 1939 experiment when harvested at 70 days. The plants are representative of 15 grown at soil temperatures shown on the pots, expressed in degrees centigrade.

### Heading

Heading was assumed to have occurred when the head had completely emerged from the sheath. The Marquis wheat grown in 1937 at a soil temperature of 22° C. headed in an average of 57 days, while that grown at 33° C. headed in an average of 51 days. The higher soil temperature in this

ease accelerated heading by 6 days. No plants headed within 48 days, the duration of the 1938 experiment.

The 1939 heading data are included in table II. The average heading time was 65 days at 22° and 54 days at 34° C., a decrease of 11 days. At 38° C. the average time to head increased to 61 days. A number of heads were sterile at this temperature. No heads were formed at 42° C. Heading was thus accelerated as the soil temperature rose from 22° to 34° C., but was retarded or prevented at higher temperatures.

## Summary

1. Marquis spring wheat was grown in the greenhouse at soil temperatures ranging from 22° to 44° C. over a period of three years.

2. Plant height, root length and extent, and tiller number decreased as the soil temperatures rose from 22° to 42° C.

3. Greatest dry weights of tops and roots and total dry weight, at time of harvest, resulted in plants grown at a soil temperature of 22° C., the lowest maintained in these experiments. The top root ratio reached a maximum at temperatures of 30° to 34° C., falling again as the soil temperature was further increased.

4. Leaves were largest at 22° C. and became lighter in color as the soil temperature rose above 32° C.

5. Increasing the soil temperatures from 22° to 34° C. accelerated heading by as much as 11 days. Soil temperatures above 34° C. retarded or prevented earing.

THE UNIVERSITY OF CHICAGO

## LITERATURE CITED

1. ALLEN, R. C. The effect of soil temperature on the growth and flowering of certain greenhouse crops. Proc. Amer. Soc. Hort. Sci. **32**: 635–637. 1934.
2. BENCHLEY, W. E. On the relations between growth and the environmental conditions of temperature and bright sunshine. Ann. Appl. Biol. **6**: 211–244. 1920.
3. BUSHNELL, J. The relation of temperature to growth and respiration in the potato plant. Minnesota Agr. Exp. Sta. Tech. Bull. 34. 1925.
4. CLEMENTS, F. E., and MARTIN, E. V. Effect of soil temperature on transpiration in *Helianthus annuus*. Plant Physiol. **9**: 619–630. 1934.
5. JONES L. H. Is soil temperature important? Florists Exch. and Hort. Trade World. Jan. 15, 1938.
6. ————————, JOHNSON, J., and DICKSON, J. G. Wisconsin studies upon the relation of soil temperature to plant disease. Wisconsin Agr. Exp. Sta. Res. Bull. 71. 1926.

7. KRAMER, P. J. Effects of soil temperature on the absorption of water by plants. Science n. s. **79**: 371–372. 1934.
8. SACHS, J. Beiträge zur Lehre von der Transpiration der Gewächse. Bot. Zeit. **18**: 121–126. 1860.
9. TAYLOR, J. W., and MCCALL, M. A. Influence of temperature and other factors on the morphology of the wheat seedling. Jour. Agr. Res. **52**: 557–568. 1936.
10. WORT, D. J. Vernalization of Marquis wheat and other spring cereals. Bot. Gaz **101**: 457–481. 1939.

# EFFECT OF CERTAIN THIOCYANATE SPRAYS ON FOLIAGE AND FRUIT IN APPLES[1]

R. B. Dustman and I. J. Duncan

(WITH ONE COLORED PLATE)

As set forth briefly in a recent publication (**5**) the authors have found that certain thiocyanates, applied as sprays to the foliage and fruit during the growing season, affect the formation of red color in apples. This red pigment has been shown (**4, 6**) to be idaein, a glycoside which yields cyanidin and galactose on hydrolysis. Growers in general recognize the importance of good color in apples, and any practical procedure which improves color may offer an advantage from the standpoint of sale value.

During the summer of 1936 amid general trials with numerous substances including both organic and inorganic materials, some of which were applied as injections, others as sprays, and still others as dips or immersion treatments, observations suggested that color was being affected in those fruits receiving sprays of soluble thiocyanates. Further trials during the following season confirmed the earlier results and indicated that the thiocyanate ion was the effective agent, irrespective of the positive ion with which it was associated. Denny (**1, 2, 3**) has already shown that sodium, potassium, and ammonium thiocyanates exhibit an unusual physiological effect on dormant potato tubers by breaking or shortening the normal rest period.

## Materials and methods

### Equipment and controls

During the summers of 1937 and 1938 all materials used were applied to individual limbs with a small hand-sprayer while nearby limbs were protected from drifting spray by sheets of heavy paperboard. In 1939 most of the applications were made with power-spray outfits. In the power-spray trials entire trees were sprayed except for one limb of each tree, which was selected as a control and covered with a heavy canvas during the spraying operation.

### Varieties

The varieties tried thus far have included Duchess, Red Duchess, Melba, McIntosh, Maiden Blush, Wagner, Delicious, Jonathan, Stayman, Rome, York, Wealthy, Winesap, Spy, Baldwin, Grimes, Golden Delicious, Yellow Transparent, Early Harvest, and several others.

[1] Published with the approval of the Director of the West Virginia Agricultural Experiment Station as Scientific Paper no. 229.

### Thiocyanates

In the hand-spray trials the substances used have included combinations of the thiocyanate radical with sodium, potassium, calcium, magnesium, barium, copper, lead, ammonium, methyl, ethylene, isopropyl, n-butyl, urea, hydrazine, aniline, pyridine, trimethyl amine, triethanol amine, nicotine, and other groups. In these trials certain of the thiocyanates were tried in mixtures with other common spray materials such as arsenate of lead, bordeaux, lime sulphur, and flotation sulphur.

In the power-spray trials sodium thiocyanate was the only thiocyanate used, and it was applied alone in all instances.

### Concentrations

The concentrations employed in the hand work have varied from 0.05 per cent. to 0.5 per cent. (in the case of certain insoluble substances suspensions of corresponding strength were used, sometimes with the addition of other materials to increase the solubility of the desired substance). The concentrations of sodium thiocyanate in the power-spray trials varied from 0.05 per cent. to 0.2 per cent.

### Time and number of applications

The number of sprays applied has varied from one to five, and the time of application has varied from June to September, inclusive, depending upon the variety under test, its stage of maturity, and the character and degree of response obtained. When more than one spray was applied, applications were usually made at intervals of approximately ten days. With late fall and winter varieties two to four sprays applied during August and early September in general gave best results.

## Results

### Effect on foliage

Virtually all of the thiocyanate sprays that have produced color effects have resulted also in a greater or lesser amount of injury to the foliage. In some instances where the materials were applied in too great concentration, or too frequently, this injury has been excessive; in other instances the amount of leaf-burn has been little or no more than would be expected from other spray materials in common use. The first noticeable effect of the spray is a marginal burn of varying width at the edges and tips of the leaves which makes itself apparent over a period of several days following the application of the thiocyanate. Later, in many leaves, particularly following additional applications, a chlorotic area or band develops inside the marginal burn, which has now become brown in appearance. Some of the

smaller and more tender leaves of the leaf clusters may turn yellow and fall. Repeated applications of spray result in an increase of the chlorotic tissue, but the portions of the leaf next the midrib remain green. Many of the leaves tend to curl slightly at the edges where the margins are brown.

An interesting feature of this chlorotic condition is its tendency to disappear as the sprays are discontinued and the season advances. Thus there is a partial recovery of the tree from the shock sustained, which in some instances has been so complete that the only abnormal appearance at the end of the season was a narrow brown strip at the margins of the injured leaves. Sprayed and unsprayed foliage was apparently almost equally green.

Another feature of interest is the observation that the foliage of the sprayed limbs or trees appears to be retained equally well in the fall with that of the unsprayed controls. No differences have been noted in the time of leaf fall.

### Effect on fruit

With most varieties any increases in the amount of red color formed do not occur suddenly but come in gradually over a period of weeks. There is a change not only in the amount of pigment produced but also in the quality of the pigment formed. Thus the normal, natural red color of the Stayman is a dark red or brick red frequently admixed with varying amounts of yellow, green, and brown. The Stayman sprayed with thiocyanates, on the other hand, shows a brighter red with a coral tint. This difference is most readily observed by a comparison of sprayed and unsprayed fruits.

In addition to the changes in red coloration there are also changes in ground color. The dark green of the unsprayed fruit is replaced in considerable degree in the sprayed fruit by the lighter greens, yellow greens, and yellows ordinarily associated with more advanced maturity. This is especially true for varieties normally showing relatively small areas of red and correspondingly larger areas of green or yellow. When sprayed with thiocyanates, Golden Delicious and Grimes may be greenish yellow or full yellow rather than green when harvested. Spraying likewise tends to increase slightly the amount of blush sometimes found on these varieties. When Yellow Transparent was sprayed with sodium or calcium thiocyanate the stage for marketing was reached several days earlier than with unsprayed fruit, largely as a result of the hastening of the color change from green to yellow. Other maturity tests might be advisable, however, before picking such fruits for market.

Although changes in ground color would seem to indicate a hastening of maturity, a corresponding progress in softening does not appear to take place. No pressure tests have been made thus far, but general observations show a firmness of tissue probably equalling that of unsprayed fruit. More-

over, retention on the tree seems to be no less for the sprayed fruit and may be somewhat greater in certain varieties. Furthermore, storage records thus far indicate a superior keeping quality for the sprayed fruit. This might be anticipated, however, since it is generally recognized that fruit of high color is less subject to scald in storage than corresponding fruit of low color.

Finally, some varieties show still further changes in the sprayed fruit. Stayman, for instance, may show a smoother surface with less scarf skin, accompanied by a moderate reduction in the size and prominence of the lenticels, and a somewhat thickened skin. Under certain conditions Grimes has taken on a waxy, yellow-amber appearance when sprayed with nicotine thiocyanate.

## Discussion

During the summer of 1939 some fifty trees were sprayed with a power-spray outfit using sodium thiocyanate, alone, at concentrations of 0.05, 0.1, or 0.2 per cent., respectively. Of these trees about thirty were located on the University Horticulture farm at Morgantown, and the remainder on the University Experiment Farm located at Kearneysville, in the eastern panhandle section near Martinsburg, West Virginia. Usually the first spray was of 0.1 per cent. concentration, and the following sprays were of 0.05, 0.1, or 0.2 per cent., respectively, depending upon the response as shown by the amount of foliage burn and color increase obtained. When only one spray was applied, particularly if this was applied rather late in the season, a concentration of 0.2 per cent. was usually employed. On the whole the results obtained at Kearneysville were not nearly as pronounced as those obtained at Morgantown. This difference in physiological response appears to be related to environmental factors, as several of the varieties tried were the same in each location.

One noticeable result of the thiocyanate spray is the difference in susceptibility to foliage burn, as shown by the different varieties. Thus the foliage of Delicious and Stayman is relatively resistant, whereas Golden Delicious and Rome are more readily injured by the spray. Color responses likewise differ with the different varieties. Stayman has responded rather better than Delicious in color effect; and Jonathan, a variety usually carrying good color in this region, nevertheless gave considerably better response than others of lower color such as Spy. It is easily possible that part of the differences experienced thus far are to be attributed to inherent individual factors since it has been observed that different trees of the same variety in the same row may show considerable variation in response to the treatment. It seems probable, however, that there are general varietal differences also.

Another feature resulting from the thiocyanate spray, and easily apparent, is the larger number of fruits showing some color and the greater amount of color on the inside branches in positions of poorer exposure to

light. With some varieties and in certain seasons normal coloration appears to be fairly good, as seen on the tree, when in reality the color is confined almost entirely to a shell of exposed fruits on the outer branches. When sprayed with thiocyanate such trees show an increased distribution of color underneath on the interior branches. This does not mean that light is unessential to color development on the sprayed fruits. On the contrary, light is a very important factor either with or without thiocyanate spray, but the latter aids in the production of color for any given conditions of exposure.

As stated earlier the increase in red color following thiocyanate sprays usually does not take place suddenly, but comes in gradually. The foliage, on the other hand, responds somewhat more rapidly. Consequently, it has been possible to gauge the concentrations and number of successive sprays desirable in any given instance as much (or more) by the leaf response as by the effect on the fruit. The thiocyanate ion appears to be a rather powerful physiological agent which affects both foliage and fruit. Further, if leaves only are sprayed and the fruits protected, or if fruits only are sprayed and the leaves protected, a color response will be obtained in the fruit in either case, but one less pronounced than where both fruit and foliage are exposed to the action of the chemical agent.

The thiocyanate spray has been applied in combination with other common spray materials including summer-strength lime sulphur, flotation sulphur, arsenate of lead, and bordeaux mixture, and appears to be compatible with each. Hand-spray trials of sodium thiocyanate *vs.* potassium thiocyanate and of sodium thiocyanate *vs.* calcium thiocyanate, in the same percentage concentration, indicated no pronounced differences in the color response obtained. More recently additional trials on similar exposures of the same tree gave no appreciable differences between solutions of equivalent thiocyanate ion concentrations of sodium, calcium, magnesium, and barium thiocyanates, respectively. Cuprous thiocyanate, and lead thiocyanate, however, were less effective in color response, possibly as a result in part of lower solubility. Ammonium thiocyanate is effective in increasing color but has the disadvantage of causing the production of small darkened areas or "pits" in the skin. These darkened areas, however, did not decrease keeping quality in storage. Among other organic thiocyanates, nicotine particularly, gave a fair color response in several varieties.

The work has not been of sufficient duration to warrant a statement of the probable effect of the thiocyanate treatment upon the bloom, set, or yield of the succeeding year's crop. In the hand-spray work individual limbs which showed moderate to severe foliage burn as a result of thiocyanate sprays in the summer of 1938, nevertheless carried good yields of fruit in 1939. It should be remembered, however, that only during the season just past have entire trees been subjected to this spray treatment, and conse-

quently evaluation of its effects on subsequent bloom and set of fruit must await further observation.

## Summary

It has been found that soluble thiocyanates, particularly inorganic thiocyanates, used as a spray on apples during the growing season, exert a pronounced physiological effect on both foliage and fruit as follows: In the foliage the leaves are subjected to spray burn and to a chlorotic condition arising from the effect of the chemical on the green coloring matter of the plant. In the fruit the amount of red color occurring normally tends to be increased and the green ground-color tends to be reduced or replaced by varying shades of yellow and yellow green.

The authors desire to express their indebtedness and appreciation to various members of the station staff, and particularly to Prof. R. S. Marsh of the Department of Horticulture, and Edwin Gould of the Department of Entomology, for generously providing facilities and cooperating in every possible manner with the work.

West Virginia Agricultural Experiment Station
Morgantown, West Virginia

## LITERATURE CITED

1. Denny, F. E. Hastening the sprouting of dormant potato tubers. Amer. Jour. Bot. **13**: 118–125. 1926.
2. ———. Second report on the use of chemicals for hastening the sprouting of dormant potato tubers. Amer. Jour. Bot. **13**: 386–396. 1926.
3. ———. Chemical treatments for controlling the growth of buds of plants. Ind. & Eng. Chem. **20**: 578–581. 1928.
4. Duncan, Ira J., and Dustman, R. B. The anthocyanin pigment of the Winesap apple. Jour. Amer. Chem. Soc. **58**: 1511–1514. 1936.
5. Dustman, R. B., and Duncan, I. J. Red color in apples. Science, n.s. **90**: 233. 1939.
6. Sando, C. E. Coloring matters of Grimes Golden, Jonathan, and Stayman Winesap apples. Jour. Biol. Chem. **117**: 45–56. 1937.

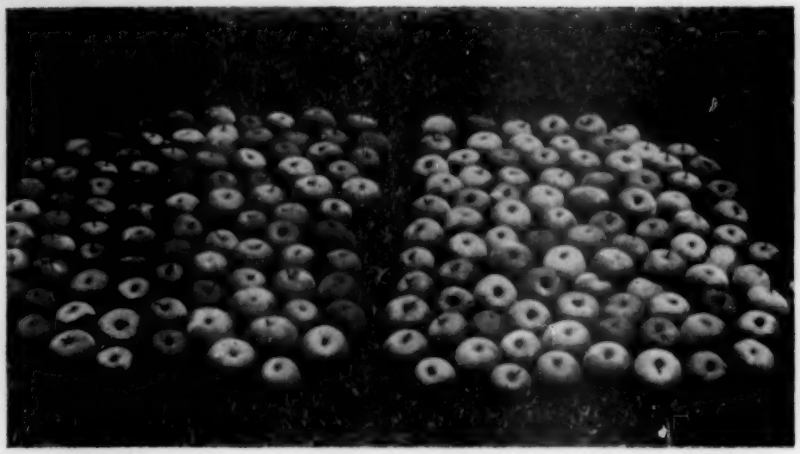


FIG. 1. Fruit from Delicious tree in Fig. 2. Control on right
Photographed Sept. 28, 1939

FIG. 2. Delicious, sprayed four times. Control limb with white tag in center
Photographed Sept. 6, 1939

DUSTMAN AND DUNCAN: THIOCYANATE SPRAYS

*Reproduced from Kodachromes*

UNIV. OF MICH.

# DOES "C.P. GRADE" SUCROSE CONTAIN IMPURITIES SIGNIFICANT FOR THE NUTRITION OF EXCISED TOMATO ROOTS?

PHILIP R. WHITE

(WITH TWO FIGURES)

Ever since the early work of KOTTE (2) and ROBBINS (4) on the limited cultivation of excised roots there has persisted the conviction, formulated at that time, that growth of such roots is dependent on unknown as well as known factors. ROBBINS (5), ROBBINS and MANEVAL (7, 8) and later ROBBINS and V. B. WHITE (10) concluded that growth of excised corn roots was dependent on some unknown material not contained in the salts of Pfeffer's solution, glucose, water, dissolved gases, peptone, or autolyzed yeast. It has subsequently been shown that continued growth of excised roots of many plants other than corn can be obtained in a nutrient containing these same salts, brewer's yeast, and sucrose (12, 14) and that roots of tomato, sunflower, and pea can be grown in a similar salt solution plus cane sugar, thiamin, and glycine, or other amino acids (17). ROBBINS and SCHMIDT (9), and FIEDLER (1) nevertheless return to this idea of unknown essentials in discussing recent results obtained with tomato roots and postulate the existence of such unknowns in the carbohydrates used.

This interpretation may, of course, be sound. But it appears not to be based on actual evidence. It should, however, be possible to test this question. If the growth obtained with certain sugars is attributable to the presence of impurities, as suggested, then progressive removal of these impurities should result in progressively poorer and poorer growth. To obtain evidence bearing on this question, a sample of sucrose *purissima* was obtained from the U. S. Bureau of Standards in Washington, D. C.[1] This is their "Standard Sample no. 17" prepared for use in molecular weight determinations, calorimetric studies, etc. Its designated properties are: specific rotation

$$[\alpha]\frac{20^\circ\text{ C.}}{546.1\text{ m}\mu} = 78^\circ .342$$

$$[\alpha]\frac{20^\circ\text{ C.}}{589.25\text{ m}\mu} = 66^\circ .529$$

when C = 26 gm. in 100 ml.; heat of combustion 3941 calories per gram weight in air; lot no. 3854; moisture less than 0.003 per cent.; ash, 0.003 per cent.; reducing substances estimated as invert sugar, 0.002 per cent. This sugar is prepared by repeated crystallization. The Bureau of Standards states: "This material is the result of many years experimentation and experience in handling and in methods of preparation.

[1] I am indebted to Dr. EUGENE PACSU, Professor of Organic Chemistry in Princeton University and specialist in the chemistry of sugars, for advice and counsel on this subject.

The impurities have been reduced to the lowest possible point consistent with the issuing of a standard sample'' (3). Spectroscopic examinations carried out in The Rockefeller Institute laboratories in New York by Dr. GEORGE I. LAVIN indicate that the inorganic impurities are largely calcium with some magnesium. Traces of calcium or magnesium are, of course, without significance (11). While an ash content of 0.003 per cent. still represents a total ash impurity of 0.6 mg. per liter of nutrient, it does not appear feasible to push the purification further at present. This paper gives the results of experiments designed to compare the effectiveness of this highly purified sucrose with that of ''C.P. grade'' sucrose such as was used in all earlier studies, with commercial cane sugar available in grocery stores, and with Pfanstiehl's ''C.P. grade'' dextrose, considered by ROBBINS and BARTLEY (6) and by ROBBINS and SCHMIDT (9) as being equally effective with sucrose as a carbohydrate source for tomato roots.

## Experimentation

Roots of this laboratory's standard clone were used for these tests. They had been grown for 301 passages in the yeast nutrient earlier employed and then for three passages in a completely synthetic (glycine-thiamin) nutrient (17), which is now used as a standard in all work. The nutrients tested contained the standard salts and accessory salts discussed elsewhere (16), 3 p.p.m. glycine (17) and 0.1 p.p.m. thiamin (15), and carbohydrate. Twenty flasks contained the usual 2 per cent. Pfanstiehl ''C.P. grade'' sucrose and represented the standard control. Twenty contained 2 per cent. commercial cane sugar purchased in a local grocery, 20 contained 2 per cent. Bureau of Standards standard-sample sucrose as described above, 20 contained 2 per cent. Pfanstiehl ''C.P. grade anhydrous'' dextrose of the same make and grade as that used by ROBBINS and BARTLEY (6) and ROBBINS and SCHMIDT (9) and, since a 2 per cent. solution of dextrose has an osmotic value twice that of a 2 per cent. sucrose solution, a fifth set of 20 was prepared containing 1 per cent. dextrose, isotonic with the sucrose solutions. Each flask contained a single root, the inocula having been carefully selected to insure uniformity. Roots were grown in these five solutions through three consecutive passages and the increment rates, numbers of branches, and general appearance compared. The series was repeated once. The results of these 600 cultures are shown in figures 1 and 2.

The numerical growth indices for the three samples of sucrose did not differ consistently or significantly either from sample to sample or from passage to passage in solutions containing the same sample, although the average index for store sugar and for Bureau of Standards sucrose was in each case about 10 per cent. greater than the control (18). Nor did the average number of branches formed differ significantly. The growth habits, however, did differ

somewhat. Roots in Pfanstiehl's "C.P. grade" sucrose and in grocery store sugar were almost identical in appearance, being rather thick, white and bent. The average maximum length of their branches was 10 mm. and 9 mm., respectively. Those grown in the Bureau of Standards sucrose were somewhat more slender and flexible, not bent, with somewhat shorter branches (7 mm.

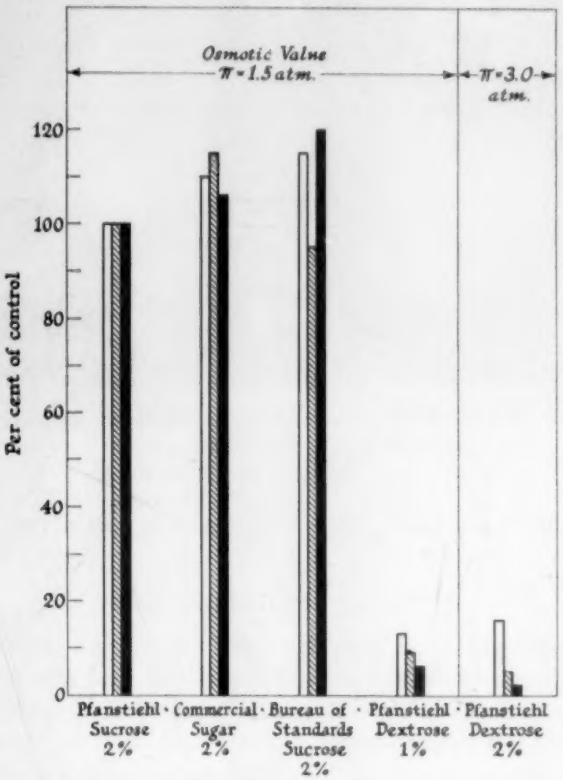



FIG. 1. Numerical indices of growth of excised tomato roots in various sugar solutions as indicated. Open columns represent the first passage, hachured columns the second passage, and solid columns the third passage in the experimental solution.

average maximum) but more regular habits. While experience has shown that, in general, thickened roots of the type formed in the first two sugars result from slightly injurious cultural conditions, the difference was not sufficiently marked to be of certain significance. Roots grown in dextrose solutions, on the other hand, were definitely inferior to those grown in any of the sucrose solutions (**18**). This last finding is contrary to the conclusions of ROBBINS and BARTLEY (**6**) and ROBBINS and SCHMIDT (9) and supports the earlier conclusions of the writer (**13**).

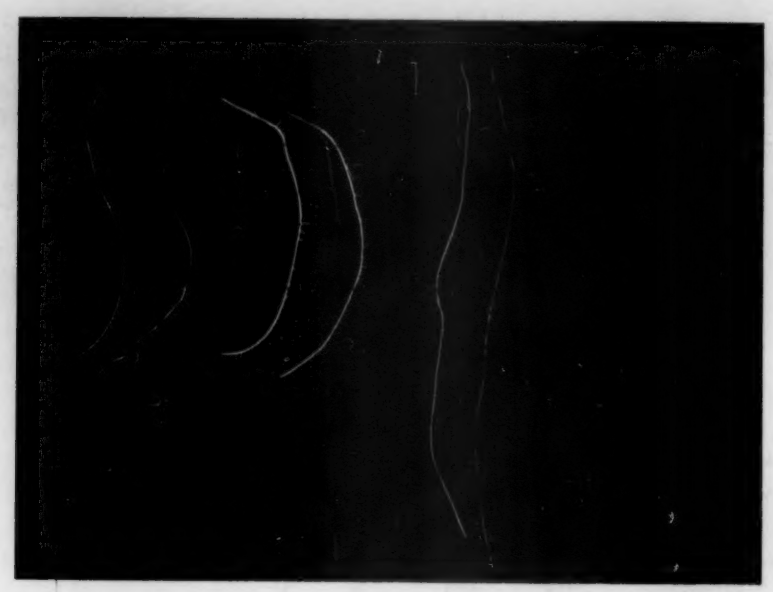


FIG. 2. The two roots at the left were grown in 2 per cent. store sugar, the set next to them in 2 per cent. Pfanstiehl "C. P. grade" sucrose, those in the middle in 2 per cent. Bureau of Standards sucrose, those next in 2 per cent. Pfanstiehl "C. P. grade" dextrose and those at the far right in 1 per cent. Pfanstiehl "C. P. grade" dextrose. The photograph was taken at the end of three passages in each solution. (Photograph by J. A. CARLILE.)

## Discussion and conclusions

From the data presented here, it is clear that growth in the most highly purified sample of sucrose was slightly superior to that obtained in the less pure samples. Pfanstiehl's "C.P. grade" sucrose may contain impurities which affect the growth of excised tomato roots, but these impurities certainly do not stimulate elongation. They tend rather to retard it. The Bureau of Standards sucrose contained about 35/1000 as much ash as did Pfanstiehl's "C.P. grade," yet supported slightly more satisfactory growth than did the latter. A differential of 35 : 1000 in known ash content had little effect on growth and that opposed to the effect expected from the theories cited above. Pfanstiehl's "C.P. grade" sucrose does not appear to be superior in this respect to the particular sample of grocery store sugar used in these tests, although no evidence has been collected as to the relative reliabilities of sugar from these two sources. The facts can be reconciled with the idea of significant impurities only if we assume that both pure and impure sugars contained the same impurities at concentrations giving the same effects. Either the postulated impurities were organic and were not fractionated by

the recrystallization process, or else they were present in quantities greatly in excess of the optimum, yet were not toxic at these high concentrations. These are rather large assumptions to make without supporting evidence. And the facts are equally well in agreement with the alternative and much simpler assumption that significant impurities were not present in these materials.

It is, therefore, concluded that the best evidence at present available does not indicate the existence in "C.P. grade" sucrose of impurities necessary for, or clearly beneficial to, the growth of excised tomato roots.

THE DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH
PRINCETON, NEW JERSEY

## LITERATURE CITED

1. FIEDLER, H. Die pflanzliche Gewebe- und Organkultur. Zeitschr. Bot. **33**: 369–416. 1938.
2. KOTTE, W. Kulturversuche mit isolierten Wurzelspitzen. Beitr. allg. Bot. **2**: 413–434. 1922.
3. NATIONAL BUREAU OF STANDARDS, Washington, D. C. Letter of February 13, 1939.
4. ROBBINS, W. J. Cultivation of excised root tips and stem tips under sterile conditions. Bot. Gaz. **73**: 376–390. 1922.
5. ———. Effect of autolized yeast and peptone on growth of excised corn root tips in the dark. Bot. Gaz. **74**: 59–79. 1922.
6. ———, and BARTLEY, MARY A. Use of dextrose by excised tomato roots. Science n.s. **86**: 290–291. 1937.
7. ———, and MANEVAL, W. E. Further experiments on growth of excised root tips under sterile conditions. Bot. Gaz. **76**: 274–287. 1923.
8. ———, and ———. Effect of light on growth of excised root tips under sterile conditions. Bot. Gaz. **78**: 424–432. 1924.
9. ———, and SCHMIDT, MARY BARTLETT. Growth of excised roots of the tomato. Bot. Gaz. **99**: 671–728. 1938.
10. ———, and WHITE, VIRGINIA B. Limited growth and abnormalities in excised corn root tips. Bot. Gaz. **98**: 209–242. 1936.
11. WHITE, P. R. Concentrations of inorganic ions as related to growth of excised root tips of wheat seedlings. Plant Physiol. **8**: 489–508. 1933.
12. ———. Potentially unlimited growth of excised tomato root tips in a liquid medium. Plant Physiol. **9**: 585–600. 1934.
13. ———. Plant tissue cultures. Bot. Rev. **2**: 419–437. 1936.

14. ————————. Cultivation of excised roots of dicotyledonous plants. Amer. Jour. Bot. **25**: 348–356. 1938.
15. ————————. Vitamin $B_1$ in the nutrition of excised tomato roots. Plant Physiol. **12**: 803–811. 1937.
16. ————————. Accessory salts in the nutrition of excised tomato roots. Plant Physiol. **13**: 391–398. 1938.
17. ————————. Glycine in the nutrition of excised tomato roots. Plant Physiol. **14**: 527–538. 1939.
18. ————————. Sucrose *vs.* dextrose as carbohydrate source for excised tomato roots. Plant Physiol. **15**: 355–358. 1940.

## BRIEF PAPERS

# SUCROSE VS. DEXTROSE AS CARBOHYDRATE SOURCE FOR EXCISED TOMATO ROOTS

PHILIP R. WHITE

In early studies on the cultivation of excised roots of wheat (3) carbohydrate was supplied in the form of "C. P. grade" dextrose (Pfanstiehl) at a concentration of 2 per cent. This choice was based on ROBBINS's earlier studies on roots of corn and was made without personal experimentation. Since the results seemed to be satisfactory, and interest at that time centered rather around other problems, no attempt was then made to determine the relative merits of other sugars. When, in 1932, attention was turned from wheat to tomato, comparably satisfactory results were not at first obtained. A survey of carbohydrate sources was made to determine if dextrose, shown to be satisfactory for wheat, might be surpassed by some other sugar. The carbohydrates tested were: one pentose (l-arabinose); five hexoses (d-mannose, d-galactose, d-glucose, d-fructose, and i-inosite); three di-saccharides (sucrose, lactose, maltose); one tri-saccharide (raffinose); one polysaccharide (dextrin); and one unidentified mixture (honey). All of these except the mannose and maltose (both Kahlbaum) and the honey were Pfanstiehl "C. P. grade" products. They were made up in nutrients containing the standard salts and yeast extract of the solution earlier developed and were introduced at concentrations equimolecular with the 2 per cent. dextrose solution previously employed. The results of that test are summarized in table I. They

TABLE I

GROWTH OF EXCISED TOMATO ROOTS IN NUTRIENTS CONTAINING EQUIMOLECULAR SOLUTIONS OF VARIOUS SUGARS

(DATA COLLECTED IN DECEMBER, 1932, AND JANUARY, 1933)

| SUGAR | MEAN GROWTH RATE MM./CULT./DAY | CONDITION |
|---|---|---|
| l-arabinose | None | Crooked, pale. No growth |
| d-mannose | 0.3 | Slender, white, bent. Good condition |
| d-galactose | 0.3 | Slender, brown, tips black. Poor condition |
| d-glucose | 0.5 | Swollen, brown, branched. Poor |
| d-fructose | 0.5 | " " " " |
| i-inosite | 0.6 | Slender, white, tips dead. Poor |
| sucrose | 3.6 | Slender, clean, white. Excellent |
| lactose | 0.6 | Slender, clean, white. Good condition |
| maltose | 0.5 | Slender, brown, tips black. Poor |
| raffinose | 0.7 | Slender, clean. Bases green. Good condition |
| dextrin | 0.7 | Slender, clean, white. Good condition |
| honey | None | Swollen, brown. Poor condition |

represent an unequivocal demonstration of the superiority of sucrose under these experimental conditions over all of the other sugars examined. A concentration of 2 per cent. sucrose was subsequently found somewhat superior to the 4 per cent. equimolecular with the 2 per cent. dextrose used for wheat. Since the major interest at that time was not to investigate carbohydrate metabolism but rather to find conditions which would permit unlimited growth, the details of these experiments were not published and the question was passed over with the statement (1934) that "sucrose . . . was found experimentally to be superior to dextrose (for tomato)" (**4**, p. 587) and (1936) that "tomato requires sucrose, being unable to utilize dextrose under the conditions studied" (**5**, pp. 430–431).

These statements stood unchallenged until 1937 and 1938 when ROBBINS and BARTLEY (**1**) and ROBBINS and SCHMIDT (**2**) concluded on the basis of their own experiments that "tomato roots *are* able to assimilate dextrose." These authors used several strains of tomato roots, including one obtained from the present writer, and used several sources of sucrose and dextrose. Their results do not show dextrose to be superior to sucrose but do show that carbohydrate source was not a limiting factor in their experiments (**2**, table 14). They are not directly comparable with results obtained in this laboratory, since a different source of yeast, a different length of culture period, and a different method of evaluating growth were employed. They are, nevertheless, sufficiently at variance with the results obtained here to make a re-examination of the question desirable. No attempt has been made to repeat the work of these authors. My own earlier experiments have been repeated using a technique similar to that which was employed before, except for minor improvements introduced from time to time during the past six years.

The roots used were of this laboratory's "Standard Clone C," also tested by ROBBINS and BARTLEY (**1**) and ROBBINS and SCHMIDT (**2**), and were in the 304th passage at the beginning of the experiments. The control nutrient was the completely synthetic solution employed as standard since June 21, 1938. This nutrient regularly gives results identical with those obtained in the earlier yeast extract medium. It contains the six salts of a modified Uspenski solution, four accessory salts, 0.1 p.p.m. thiamin and 3 p.p.m. glycine, and 2 per cent. Pfanstiehl "C. P. grade" sucrose. Its osmotic value is $\pi = ca.$ 1.5 atm. Four solutions were compared with this, all identical except for the carbohydrate. These were made up with (1) 2 per cent. commercial sugar bought in a local grocery; (2) 2 per cent. Bureau of Standards specially purified sucrose (**6**); (3) 2 per cent. Pfanstiehl "C. P. grade anhydrous" dextrose as nearly identical with that used by ROBBINS and BARTLEY and ROBBINS and SCHMIDT as obtainable, and giving an osmotic value of $\pi = ca.$ 3.0

atm.; and (4) 1 per cent. Pfanstiehl "C. P. grade anhydrous" dextrose having an osmotic value the same as that of the sucrose solutions. Cultures were grown in the laboratory used for all of the writer's work of the past six years, in diffuse daylight. Twenty cultures were grown in each solution, the experiments were carried through three passages, and the entire series was repeated once. The results of these 600 cultures are presented in table II (see

TABLE II

RELATIVE GROWTH RATES OF EXCISED TOMATO ROOTS IN NUTRIENTS CONTAINING SUGARS OF VARIOUS SORTS, CONCENTRATIONS, AND DEGREES OF PURITY

| SUGAR | INCREMENT AS PERCENTAGE OF CONTROL | | | |
|---|---|---|---|---|
| | PASSAGE | | | AVERAGE |
| | 1 | 2 | 3 | |
| Sucrose Pfanstiehl C. P. 2 per cent. | 100 | 100 | 100 | 100 |
| Sucrose Bureau of Standards 2 per cent. | 115 | 95 | 120 | 110 |
| Sucrose Commercial 2 per cent. | 110 | 115 | 106 | 110 |
| Dextrose Pfanstiehl C. P. 1 per cent. | 13 | 9 | 6 | 9 |
| Dextrose Pfanstiehl C. P. 2 per cent. | 16 | 5 | 2 | 8 |

also 6, figs. 1 and 2). While growth in the three samples of sucrose was excellent and did not differ significantly from the control nor diminish consistently from passage to passage, that in both concentrations of dextrose was extremely poor—less than 10 per cent. of that in the control nutrient—and it decreased in 2 per cent. dextrose from 16 per cent. in the first passage to 2 per cent. in the third, and in 1 per cent. dextrose from 13 per cent. in the first passage to 6 per cent. in the third.

The result of this experiment thus agrees with that obtained in 1933. Under the experimental conditions used as standard in this laboratory for the past six years, sucrose is superior to dextrose as a source of carbohydrate for excised tomato roots. Since the experiments were carried out with the same brand and quality of sugars and with the same strain of roots as were used by ROBBINS and BARTLEY (1) and ROBBINS and SCHMIDT (2), the discrepancy

between the results of these authors and those here presented remains unexplained.

THE DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH
PRINCETON, NEW JERSEY

## LITERATURE CITED

1. ROBBINS, W. J., and BARTLEY, MARY A. Use of dextrose by excised tomato roots. Science n.s. **86**: 290–291. 1937.
2. ————————, and SCHMIDT, M. B. Growth of excised roots of the tomato. Bot. Gaz. **99**: 671–728. 1938.
3. WHITE, P. R. Influence of some environmental conditions on the growth of excised root tips of wheat seedlings in liquid media. Plant Physiol. **7**: 613–628. 1932.
4. ————————. Potentially unlimited growth of excised tomato root tips in a liquid medium. Plant Physiol. **9**: 585–600. 1934.
5. ————————. Plant tissue cultures. Bot. Rev. **2**: 419–437. 1936.
6. ————————. Does "C. P. Grade" sucrose contain impurities significant for the nutrition of excised tomato roots? Plant Physiol. **15**: 349–354. 1940.

# KUNDT'S RULE

G. MACKINNEY

The writer recently found (**2**) an exception to Kundt's rule, with dichloroethane and acetone as solvents for chlorophyll. EGLE (**1**) objects to dichloroethane on the ground that pheophytin has been formed. The objection cannot be sustained in the writer's experiments for two reasons; first, that with this solvent, freshly purified and distilled, chlorophyll solutions are quite stable, and show negligible change after several hours if kept in the dark. The evidence, both chromatographic and spectroscopic, (**3**) is conclusive against such a change. Second, had such a change occurred, it would have yielded a result predictable from Kundt's rule, because the pheopytin maximum is at a slightly longer wave length than that of chlorophyll.

The results in a series of solvents tested by EGLE are in no way challenged, nor need the point be belabored, but for the assumption of some writers that it is permissible to extrapolate results with organic solvents and extracted chlorophyll, in order to predict the situation in the leaf. Fortunately, active interest in the chloroplastin complex will soon preclude the necessity for discussion of the rule.

DIVISION OF FRUIT PRODUCTS
UNIVERSITY OF CALIFORNIA

## LITERATURE CITED

1. EGLE, K. Über die Gültigkeit der Kundtschen Regel bei Chlorophyll. Sitz. Heidelberg Akad. Wiss. Klasse Math.-naturwiss. Abh. **1**: 19–30. 1939.
2. MACKINNEY, G. Applicability of Kundt's rule to Chlorophyll. Plant Physiol. **13**: 427–430. 1938.
3. ———. Some absorption spectra of leaf extracts. Plant Physiol. **13**: 123–140. 1938.

## NOTES

**New England Section.**—The New England Section will hold its annual meeting at Dartmouth College, Hanover, New Hampshire, on May 10–11, 1940. These meetings again will offer the opportunity for plant scientists in the area to participate in the programs of discussion and to add to their lists of friendships. Dr. CHARLES J. LYON of Dartmouth will have charge of the arrangements.

In addition to the papers to be presented on other subjects, several workers in the field of growth physiology will participate in a symposium on Growth Control in Trees. The flexibility of the program is such that groups readily form to discuss informally problems of common interest.

The annual dinner will be followed by pictures and stories of life in the North Country. All plant physiologists in the New England states will be cordially welcome to share in the benefits and privileges of the meeting.

**Western Section.**—The summer meeting of the American Society of Plant Physiologists and the meeting of the Western Section will be held at Seattle, Washington, from Tuesday, June 18, to Saturday, June 22, 1940. The program will include three symposia:

1. Photosynthesis, jointly with the Botanical Society of America.
2. Aquatic Botany, also jointly with the Botanical Society of America.
3. Phosphate Nutrition, jointly with the Society of Soil Scientists.

Three half-day sessions are reserved for the reading of short papers, and also one for papers having a bearing on horticulture, jointly with the Society for Horticultural Science.

On Saturday, June 22, an excursion to the Oceanographic Laboratories of the University of Washington at Friday Harbor has been planned. This provides an excellent opportunity to see the beautiful San Juan Archipelago with its amazing growth of algae such as the large Nereocystis, in addition to the outstanding laboratories.

**Southeastern Section.**—It is gratifying to be able to report that the Southeastern Section of the A.S.P.P. has completed its permanent organization. In a meeting held in connection with the Association of Southern Agricultural Workers at Birmingham, Alabama, on February 7, 1940, permanent officers were elected for the year 1940–41. Dr. CLAIR L. WORLEY, the University of Georgia is chairman of the Section, and *ex-officio* member of the executive committee of the national organization; Mr. DAVID D. LONG, Director of Agricultural Research of the International Agricultural Corporation, Atlanta, Georgia, was elected vice-chairman; and Dr. THOMAS J. HARROLD, University of Georgia, was elected secretary-treasurer. These

officers will draw up a constitution and set of by-laws to be presented for consideration at the 1941 meeting.

The Southeastern Section will continue its meetings with the Association of Southern Agricultural Workers annually in February. It has become affiliated with this Association, therefore, and at a later time will present a request for modification of its territorial limits to conform to that of the Association. This would require the addition of three or four states to the territory of the Section.

A membership committee has been organized with Dr. GEORGE M. ARMSTRONG, Clemson College, chairman. It seems certain that a larger membership in A.S.P.P. in the South will result from the activities of the Section. The 1940 meeting was attended by 14 men representing seven states, Virginia, North Carolina, South Carolina, Georgia, Alabama, Mississippi, and Louisiana. Three scientific papers were read, one on carotene and vitamin C by O. A. LEONARD; one on nutrient deficiencies in pine seedlings by L. J. PESSIN; and one on Nielsen's substance B by C. L. WORLEY. Those in attendance were enthusiastic over the privileges and opportunities afforded by organization. We predict a very bright future for this Section.

**Changes in Proofs.**—At the Columbus meeting the A.S.P.P. finally took official action on the matter of excessive changes made by authors in their proofs. Such changes may be defined as eliminations, additions, or changes in wording or punctuation not found in the original manuscript as submitted. It has been ascertained by statistical study that a change of 2 per cent. of the lines is an average change, actually achieved in some entire numbers of the journal. Any more than this will be considered excessive.

The editor has been authorized to have the costs for excessive changes in the proofs added to the author's bill for reprints. The charge is 12 cents per line. In administering this regulation, very simple rules are desirable. Authors will be allowed an average change of one line per page, which is just slightly more than 2 per cent. The printer's records will be used to determine how many lines have been changed; and when the author's line changes exceed one per page of printed text, the excess changes at 12 cents per line will be added to the bill for reprints. The majority of authors will find that this regulation causes them no extra expense. The few who do not properly polish their manuscripts before submitting them, or who change their minds about interpretations before the papers can be printed, should pay for the extra work they demand.

**Corresponding Members.**—Election of corresponding members to the American Society of Plant Physiologists is strictly limited. In order to provide complete information concerning the present roster of Corresponding Members, the list is printed in full, with date of election.

FREDERICK F. BLACKMAN, University of Cambridge, 1932.
GOTTLIEB HABERLANDT, University of Berlin, 1934.
BOHUMIL NĚMEC, Charles University, Prague, 1935.
NICOLAI A. MAXIMOV, Saratov, 1935.
HENRY H. DIXON, Trinity College, Dublin, 1937.
ALFRED URSPRUNG, University of Freibourg, 1937.
SIR JOHN RUSSELL, Rothamsted Experimental Station, 1938.

Three other great plant physiologists have been elected, but have died since election. These are:

F. A. F. C. WENT, University of Utrecht, 1932; deceased 1935.
V. N. LUBIMENKO, Leningrad, 1934; deceased 1937.
HANS MOLISCH, University of Vienna, 1935; deceased 1937.

**Program Committee.**—The program committee for the seventeenth annual meeting at Philadelphia has been appointed by President JOHN W. SHIVE, as follows:

Dr. J. T. CURTIS, the University of Pennsylvania.
Dr. W. R. ROBBINS, New Jersey Agricultural Experiment Station.
Dr. WILLIAM SEIFRIZ, chairman, the University of Pennsylvania.

With this able leadership, and with cooperation of all members of the Society it should be possible to make this meeting a memorable occasion. Those who wish to participate in the sessions can aid the committee very materially by early submission of titles. If there is sufficient demand for time on the program, parallel programs of non-competing subjects will be arranged. Suggestions from the members are always welcome.

**Frank Marion Andrews.**—At the sixteenth annual meeting of the American Society of Plant Physiologists it was voted to dedicate one of the numbers of Plant Physiology for 1940 to Dr. FRANK MARION ANDREWS, who will celebrate the seventieth anniversary of his birth on July 27, 1940. Dr. ANDREWS was born at Vienna, a small village in Scott County, Indiana, on July 27, 1870. He was educated in the public schools of Indiana, and after completing his secondary education, he entered Indiana University, with which he has been identified for half a century. He received his A.B. degree at Indiana in 1894, and the A.M. in 1895. He became an assistant in botany at I. U. in 1894, a position he held until 1897, when he was given the rank of instructor. During his instructorship, 1897–1904, he took leave long enough to win his Ph.D. degree at Leipzig in 1902. He spent a summer at the Marine Biological Laboratory, Woods Hole, Massachusetts, in 1903. In 1904 he was promoted to an assistant professorship, a position he held until 1907. It was during this period that he met Miss MARIE OPPERMAN, a botany major at Indiana University, afterwards a student with Dr. MARGARET C. FERGUSON at Wellesley for an advanced degree. They were married on June 17,

1907. Dr. ANDREWS was promoted to an associate professorship in 1907, a rank which he held until 1922, when he was advanced to a professorship. This position he still holds, for he reaches the usual retiring age with one more year of service. It is unusual indeed for anyone to be identified with a single institution for so many years.

As a teacher, Professor ANDREWS has an enviable reputation as one of the best on the I. U. campus. His class work was always well organized, and went along so smoothly that students accomplished a large amount of work without strain. The spirit of his class rooms and laboratories was one of jovial comradeship in the work to be accomplished; and students loved and respected his sensitive feeling for how much and how well the work should be done. They could not bear to fail him; work, spiced with good humor and pithy admonitions, was pleasure even in oppressively hot summer weather. Students learned much besides the regular prescribed work.

His personality, his thorough knowledge of his subject and of other fields of interest, his unfailing sense of humor, his personal oversight of the preparation for work, his kindly personal interest in all students, bright or otherwise, and his keen desire to see each one succeed, are the elements of his success as a teacher. A student would not need to be told more than once that: "A college education for some, is a four-year attempt to keep from getting what one comes to college to get!" When the tension of work reached the danger point, a pleasant joke brought relaxation and renewed zest for labor.

Professor ANDREWS belongs to numerous scientific organizations, was president of the Indiana University chapter of Sigma Xi in 1916, is a fellow in the Indiana Academy of Science, which organization he served as vice president in 1920, and president in 1921. He belongs to the Soc. Linn. de Lyon. His interest in the American Society of Plant Physiologists ever since its organization has been demonstrated in many services rendered. In 1935 he was awarded the CHARLES REID BARNES life membership in recognition of his long and valuable service to plant physiology. In 1937 he demonstrated his abiding interest in the Society's welfare by becoming one of its patrons at the Indianapolis meeting. In a way, the Society represents to him a dream come true, in what it is accomplishing for the welfare of plant physiology and plant physiologists.

Among his priceless memories are the privileges of association with Dr. GEORGE J. PEIRCE, who was Assistant Professor at Indiana during the last two years of his assistantship in botany ('95–'97), and with the great leaders in Germany, Holland, and Italy during his European visits. After his degree had been awarded in 1902, he had visited Amsterdam in 1907, Leipzig in 1907–1908, the Naples Station in 1908, and Strassburg in 1908–1909. He occupied the Smithsonian table at Naples. The inspiration of tireless men who aided him and supplied his needs for research during these "Wander-

jahre'' has lived in his heart and mind, and has helped to set the pattern of his life. More than a hundred contributions to the literature of plant physiology attest his devotion to research.

That he lives the principles of democracy has been demonstrated in his recent illness. None have been too humble to remember him. People in all walks of life, those on relief, day laborers, and janitors, as well as professors and bankers, rich and poor, regardless of color, have united in sympathetic interest in his welfare. Such tribute comes to those whose lives bear the stamp of truly democratic ideals.

The photographs reproduced in this issue of PLANT PHYSIOLOGY were prepared by the Shaw Studio, Bloomington, Indiana. The fireside portrait was enlarged from a small photograph taken by Mr. SWANSON, a photographer of the U. S. Navy. We appreciate the opportunity and privilege of reproducing them.

On behalf of the members of the American Society of Plant Physiologists, we extend congratulations and good wishes to Professor and Mrs. ANDREWS on the occasion of the seventieth birthday anniversary. We hope that all signs of ill health may vanish and that both may enjoy many more years together.

**Minor Elements.**—The following announcement has been sent to Plant Physiology by Mr. HERBERT C. BREWER, Director of the Chilean Nitrate Educational Bureau, Inc., 120 Broadway, New York City.

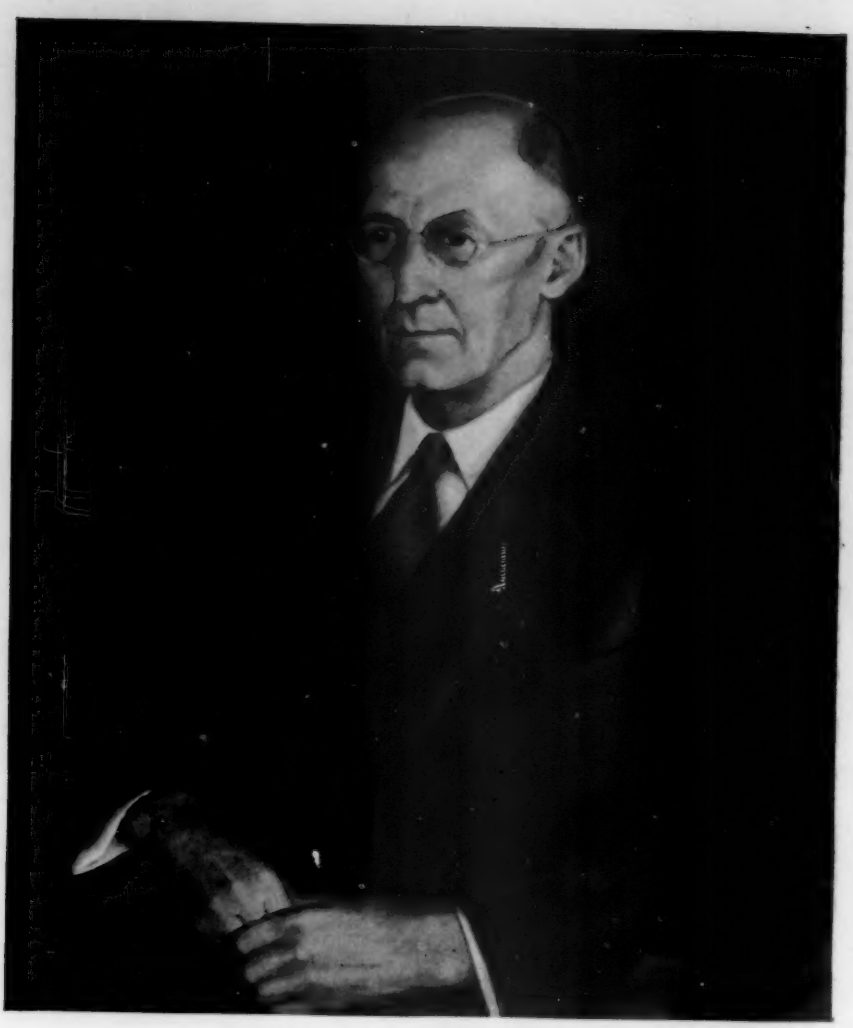
"The third edition of the *Bibliography of References to the Literature on the Minor Elements,* published Feb. 1, 1939, contained 4,628 abstracts and references, in a volume of 488 pages."

"Owing to its size, it is not practical to continue publication of complete editions of the bibliography, especially since the volume of material becoming available makes it desirable to publish more frequently. Accordingly Supplement No. 1 to be published shortly will contain about 700 abstracts and references, noted since publication of the third edition."

"It is planned hereafter to publish supplements at intervals of approximately one year."

"A botanical index is now available for the third edition, and is also being included in the supplement."

It is unnecessary to emphasize the importance of this announcement, or of the service rendered to plant physiologists and to agriculture generally by this program of publishing annual supplements to this important Bibliography. Every member of the American Society of Plant Physiologists should avail himself of the opportunity to obtain these supplements as they appear. The botanical index will add immensely to the value of the third edition. The inauguration of this high grade service to scientists reflects great credit upon the Bureau and its Director.



CHARLES FREDERICK HOTTES
JULY 8, 1870

UNIV. OF MICH.

THIS NUMBER OF PLANT PHYSIOLOGY

IS DEDICATED TO

CHARLES FREDERICK HOTTES

IN CELEBRATION OF

THE SEVENTIETH ANNIVERSARY OF HIS BIRTH

JULY 8, 1870

THIS NUMBER OF PLANT PHYSIOLOGY

IS DEDICATED TO

CHARLES FREDERICK HOTTES

IN CELEBRATION OF

THE SEVENTIETH ANNIVERSARY OF HIS BIRTH

JULY 8, 1870

CHARLES FREDERICK HOTTES
JULY 8, 1870

UNIV. OF MICH.